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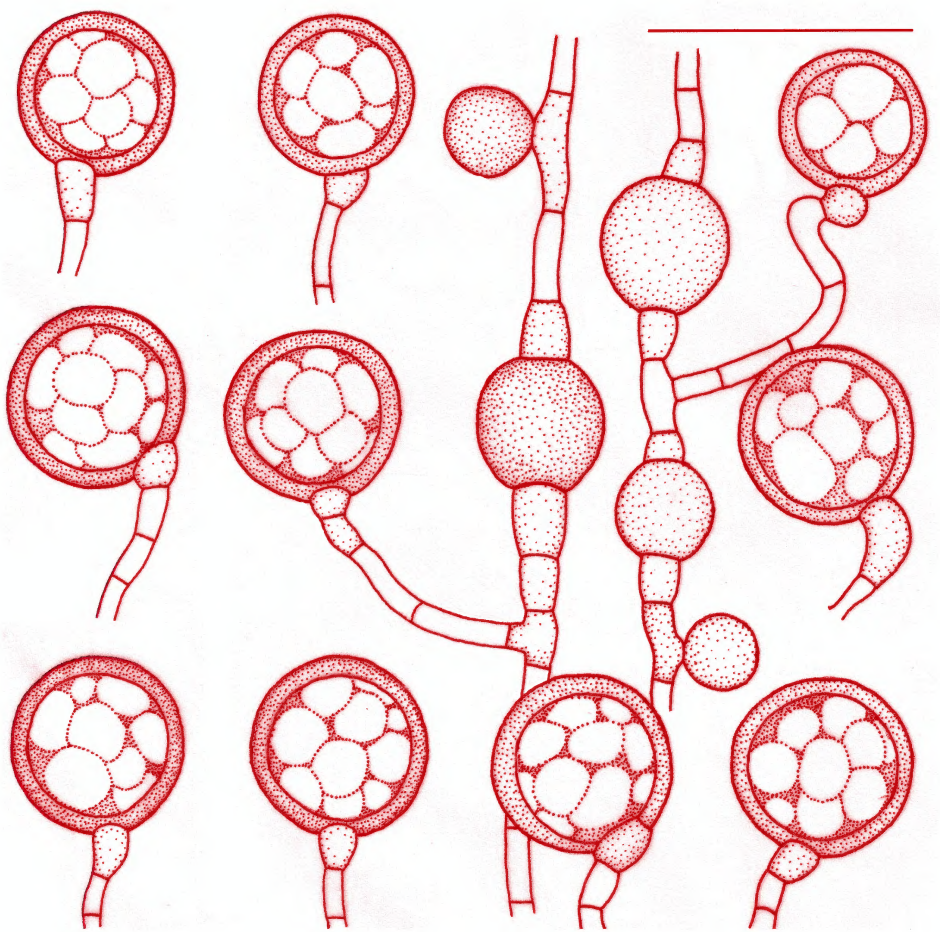

Humicola chlamydospora sp. nov.
(Wu & Zhang— FIG. 1, p. 172)
YUE-MING WU, *artist*

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PUBLICATION DATE FOR VOLUME ONE HUNDRED TWENTY-ONE
MYCOTAXON *for* July–September, volume 121 (i–xii + 1–502)
was issued on January 8, 2013

Errata from previous volumes

Volume 121

p. v, line 19	FOR: Ling-ming He,	READ: Ling-min He,
p. vii, line 15	FOR: nephrosteranic	READ: nephrosterinic
p.3, table footnotes	FOR: sporocarps	READ: basidiomata
p.5, footnote line 2	FOR: hymenodermioid	READ: hymenodermoid
p.7, Fig. 1 legend, line 3	FOR: section.).	READ: section).
p.10, line 2nd from bottom	FOR: Alnus incana	READ: *Alnus incana*
p.17, line 26	FOR: IB1998/0446	READ: IB19980446
p.199, line 3	FOR: Ling-ming He,	READ: Ling-min He,
p.233, lines 21 & 33	FOR: Henkel et al. 2011	READ: Henkel et al. 2012
p.238, line 4	FOR: (Smith et al. 2011)).	READ: (Smith et al. 2011).
p.268, line 6	FOR: *Leucoagaricus cretaceus*	READ: *Leucocoprinus cretaceus*
p. 394, line 16	FOR: recognized seven genera	READ: recognized eight genera
p.409, line 9	FOR: *columbiana*	READ: *colombiana*

Reviewers — volume one hundred twenty-two

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this volume.

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From the Editor-in-Chief

Mycotaxon 122 — Closing our 'book' on 2012, the October-December volume proposes one new genus (*Ticosynnema*) from Costa Rica and names species new to science representing *Agaricus* from Thailand; *Arthrinium* from Norway; *Boletus, Minimelanolocus,* and *Mycena* from Portugal and Spain, *Calonectria, Clitopilus, Coccomyces, Ellisembia, Humicola, Hymenochaete, Hypoderma, Hypoxylon, Polyporus, Stigmella, Terriera,* and *Tuber* from China; *Caloplaca* from India; *Coltricia* from Australia; *Dendrothele* and *Meliola* from Brazil; *Minimelanolocus* from Portugal; *Passalora* from Puerto Rico; *Puccinia* from Iran; and *Scopinella* from Argentina.

In addition to range extensions of previously named taxa, we also offer regional monographs (most with keys) on *Corticiaceae* on living bark (posthumously published by Rodrigues and Guerrero) and resupinate polypores in Brazil, *Geastrum* in Argentina, *Leptogium* in China, *Leucodecton* and *Myriotrema* in India, and *Scleroderma* in Pakistan.

One paper's multigene phylogenies support a recent morphologically based reclassification of the *Gigasporales.* Another explores phylogeny and a newly discovered sexual state for *Talaromyces flavovirens* comb. nov. while a third examines development in *Lysurus* from egg to fully hatched and a fourth provides a fascinating review of myxomycetes from fossil to Eppendorf tube.

A special thanks to four UC Berkeley authors who have solved a mystery that has puzzled this editor for over 30 years: how does one differentiate two *Suillus imitatus* varieties, *Suillus caerulescens,* and *S. ponderosus* in the field? Placing the two varieties into synonymy with an explanation of how one might separate *S. caerulescens* from *S. ponderosus* without recourse to DNA work is a restful and much appreciated outcome indeed!

Mycotaxon 123 — We are pleased to report that the first 2013 volume should be only slightly delayed. Revisions of final submissions for the next volume will begin in a few days, and we hope — somewhat optimistically — to publish the January–March volume in April.

Warm regards,

Lorelei Norvell (*Editor-in-Chief*)
26 February 2013

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Hypoxylon from China – 2: *H. dengii* sp. nov. and *H. crocopeplum* new to China

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Abstract—*Hypoxylon dengii* (*Xylariales*, *Xylariaceae*) is described from China as a new species, and *H. crocopeplum* is reported for the first time from the Chinese Mainland. The morphological descriptions and photographs of stromata and microstructures are provided based on the Chinese collections.

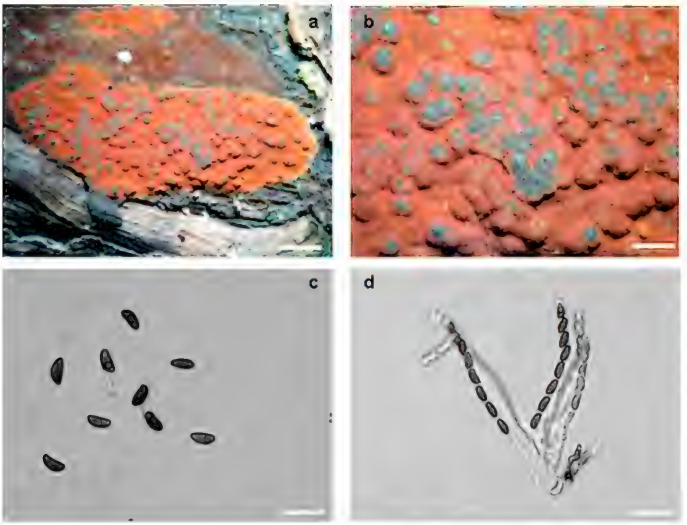
Key words—*Ascomycota*, pyrenomycetous fungi, taxonomy

Introduction

The members of the genus *Hypoxylon* are important components of forest ecosystems, where they grow on wood and decompose woody structures (Merrill et al. 1964, Rajagopalan 1966, Rogers 1979, Sutherland & Crawford 1981, Whalley 1985, Chapela & Boddy 1988, Wei et al. 1992). Publications dealing with descriptions and illustrations of *Hypoxylon* species from China are scanty (Teng 1963, Tai 1979, Abe & Liu 1995). A further study of this genus has yielded one species new to science and another new to the Chinese Mainland. Illustrated descriptions of these two species are provided in this paper.

Materials & methods

The studied specimens are deposited at the Herbarium of Mycology of Jilin Agricultural University (HMJAU). Microscopic features and measurements were made from slide preparations mounted in water, 10% KOH, and Melzer's iodine reagent. The photographs of asci, ascal apical ring, and ascospores were taken by using a VHX-600E microscope of the Keyence Corporation. The photographs of stromatal surface were taken with a ZSA30w microscope and S70 Canon camera. External stromatal colors were recorded and coded according to Rayner (1970). The methods of collecting, preservation, and identification of the examined specimens follow Ju & Rogers (1996).



Pl. 1 *Hypoxylon dengii*: a. Stromata; b. Stromatal surface; c. Ascospores; d. Asci. Scale bars: a = 0.5 mm, b = 0.2 mm, c = 15 μm, d = 20 μm.

Taxonomy

Hypoxylon dengii H.X. Ma, Lar.N. Vassiljeva & Yu Li, **sp. nov.** Pl. 1.

MycoBank MB 563782

Differs from *Hypoxylon jecorinum* by its larger ascospores and straight germ slit and from *H. crocopeplum* by its usually indehiscent perispore, smaller ascospores, and straight germ slit.

Type: China, Guangdong Province, Chebaling Nature Reserve, on the bark of a fallen branch, 26 June 2010, He S.-H. (**Holotype**, HMJAU 22432).

Etymology: The species is named in honor of Prof. Shu-Qun Deng, the first Chinese mycologist, who reported *Hypoxylon* from China.

Stromata effused-pulvinate, plane, with conspicuous perithecial mounds, 0.5–1 mm thick; surface yellow brown to rust, orange granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange to scarlet; the tissue below the perithecial layer black, inconspicuous. Perithecia ovoid to short tubular, 0.1–0.3 × 0.2–0.6 mm; ostioles lower than the stromatal surface. Asci 65–80 × 5.5–7 μm long in the spore-bearing portions, the stipes 28.5–58.5 μm long, with apical ring bluing in Melzer's iodine reagent, discoid, 0.5–1 μm high × 1–1.5 μm broad. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly to broadly rounded ends, (10–)10.5–11.5(–12.5) × 5–6.5 μm, with straight germ slit slightly less than spore length; perispore infrequently dehiscent in 10% KOH.

Additional specimen examined: **China**, **Yunnan Province**, Kunming, on the bark of a fallen branch, 2 September 2010, Ma H.-X. (HMJAU 22624).

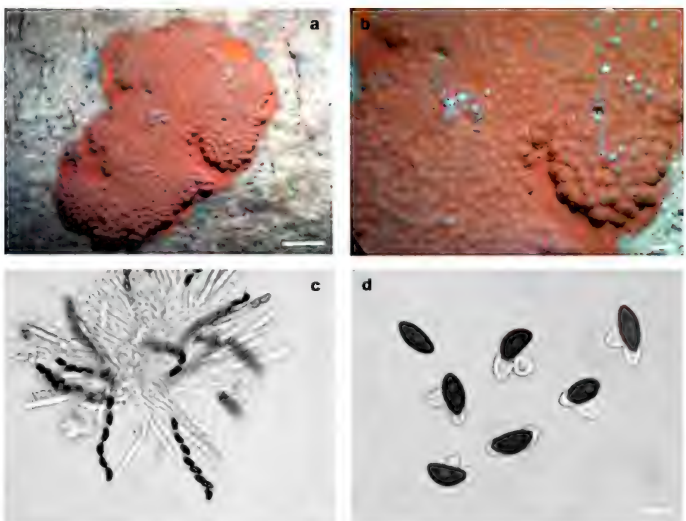
Comments — *Hypoxylon dengii* differs from *H. jecorinum* Berk. & Ravenel, which has smaller ascospores (8–9.5 × 4–5 µm) and a straight or slightly sigmoid germ slit (Ju & Rogers 1996).

Another similar species, *H. crocopeplum*, is distinguished by the dehiscence of almost every ascospore in 10% KOH, a larger ascospore size (see below), and a straight or slightly sigmoid germ slit.

In stromatal morphology and KOH-extractable pigments, *H. dengii* is also resembles *H. subcrocopeplum* Y.M. Ju & J.D. Rogers, which has longer narrower spores (11–15 × 4.5–5.5 µm) and an indehiscent perispore (Ju & Rogers 1996).

Hypoxylon crocopeplum Berk. & M.A. Curtis, Grevillea 4: 49. 1875. Pl. 2.

Stromata effused-pulvinate, plane, with conspicuous perithecial mounds, 0.4–2.5 cm long × 0.2–1.5 cm broad × 0.5–1 mm thick; surface yellow brown to rust, orange-red granules immediately beneath surface and between perithecia, with orange KOH-extractable pigments; the tissue below the perithecial layer black, inconspicuous. Perithecia ovoid, 0.1–0.3 × 0.2–0.5 mm; ostioles lower than the stromatal surface. Asci 77–95 × 8–10 µm long in the spore-bearing parts, the stipes 55–70 µm long, with apical ring bluing in Melzer's iodine reagent, discoid, 0.5–0.8 µm high × 0.8–1.5 µm broad. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with broadly rounded ends, (11.5–)12–14(–14.5) × 6–7.5 µm, with straight or sigmoid germ slit spore-length; perispore dehiscent in 10% KOH.



Pl. 2 *Hypoxylon crocopeplum*: a. Stromata; b. Stromatal surface; c. Asci; d. Ascospores. Scale bars: a = 4 mm, b = 1 mm, c = 15 µm, d = 10 µm.

Specimens Examined: **CHINA**, **Guangzhou Province**, Chebaling Nature Reserve, on the bark of a fallen branch, 26 June 2010, Ma H.-X. (HMJAU 20215); **Yunnan Province**, Mengla County, on the bark of a fallen branch, 14 August 1994, Liu P.-G. (HKAS 28660).

Comments — *Hypoxylon crocopeplum* is widely distributed in the tropics and subtropics and has been reported in many areas including Africa, Australia, Brazil, India, Mexico, U.S.A, Thailand, and Taiwan. Our collections match the eastern North American type in thin stromata with inconspicuous black basal tissue and conspicuous perithecial mounds. Miller (1961) and Ju & Rogers (1996) considered some *H. crocopeplum* specimens to have thicker stromata with tubular to long tubular perithecia and a conspicuous black basal tissue beneath the perithecial layer, while Hsieh et al. (2005) felt that such specimens represented *H. polyporoideum* Berk. ex Cooke.

Hypoxylon crocopeplum somewhat resembles *H. cinnabarinum* (Henn.) Y.M. Ju & J.D. Rogers in stromatal morphology and *H. subcrocopeplum* in stromatal morphology and KOH-extractable pigments. However, *H. cinnabarinum* has nearly equilateral ascospores and a perispore that is usually indehiscent in 10% KOH (Ju & Rogers 1996), and *H. subcrocopeplum* ascospores are indehiscent. *Hypoxylon crocopeplum* is also somewhat similar to *H. jecorinum*, which has smaller ascospores 8–9.5(–11) × 4–5 μm.

Acknowledgments

We greatly appreciate Dr. A.J. Whalley of Liverpool John Moores University and Dr. Shuang-hui He of Institute of Microbiology, Beijing Forestry University for reviewing presubmitted manuscript and giving helpful comments and suggestions. This study was supported by the National Natural Science Foundation of China (grant no. 30770005) and the Start-up Costs for Doctors in Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences. We are also grateful to Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences for funding in the project entitled "The Project of the Basic Scientific Research by the Central Public Welfare Institutes". We thank Dr. Pei-gui Liu (Kunming Institute of Botany, Chinese Academy of Sciences) who collected the fungus. We also thank Dr. Hua Peng and Dr. Li-song Wang (Kunming Institute of Botany, Chinese Academy of Sciences), for loan of specimen.

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Corticioid basidiomycetes on the bark of living trees from Porto Alegre, Brazil[1]

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Abstract —The species of *Corticiaceae* s.l. growing on the bark of living trees in the Metropolitan Region of Porto Alegre, Rio Grande do Sul State, Brazil, were studied. *Dendrothele syspora* is described as a new species, based on morphology and development of spores, and the new combination *Dendrodontia bispora* is proposed. Five species of *Dendrothele* and one of *Xenosperma* represent new records for Brazil. Descriptions, illustrations, and an identification key are included.

Key words — *Aphyllophorales*, new taxa, systematics, wood decay fungi

Introduction

Corticiaceae s.l. includes lignicolous fungi with many species producing basidiomata that develop on dead branches or woody material lying on the ground. However, it also includes species that develop on bark of living trees. Corticioid species were first reported from Rio Grande do Sul State (Brazil) by Rick (1959). Some of his materials were reviewed by Hjortstam & Ryvarden (1982) and Rajchenberg (1987). Since then, only Maluf & Guerrero (1993), Nietiedt & Guerrero (2000), and Sobestiansky (2005) have contributed significantly to this mycota.

In this work we present and discuss species belonging to genera that occupy a specialized habitat, i.e., the exposed bark of living trees: *Dendrodontia* Hjortstam & Ryvarden, *Dendrothele* Höhn. & Litsch., and *Xenosperma* Oberw.

[1] From the PhD thesis by Rodrigues, who had almost completed this paper with Guerrero shortly before his untimely death. † Deceased, 18 December 2008. ††Deceased 28 February 2012

[2]The paper presented here was prepared for publication by Vagner G. Cortez and Juliano M. Baltazar.

Materials & methods

The species treated are part of a survey of *Corticiaceae* s.l. carried out in four hills located within 60 km of one another. Except for *Dendrodontia bispora*, which was collected in an urban park, all collections were gathered in native forests surrounding Porto Alegre municipality (Rio Grande do Sul State, Brazil) during 2001–04. The vegetation in the sampled areas consists of seasonal semi-deciduous forests with 10–20 m tall hygrophilous to mesophilous communities. Specimens, analyzed macro- and microscopically following usual techniques, are deposited in the herbarium ICN (Universidade do Rio Grande do Sul, Departamento de Botânica).

Taxonomy

Key to species of *Corticiaceae* s.l. on bark of living trees in the Metropolitan Region of Porto Alegre, Rio Grande do Sul, Brazil

1a. Hyphal system dimitic ..... ***Dendrodontia bispora***
1b. Hyphal system monomitic 2
2a. Cystidia present 3
2b. Cystidia absent 4
3a. Cystidia capitate ..... ***Dendrothele capitulata***
3b. Cystidia fusiform ..... ***Dendrothele alliacea***
4a. Basidiospores asteriform, with 3 or more projections 5
4b. Basidiospores different 6
5a. Dendrohyphidia present, hyphae 1.5–2 µm diam. ..... ***Dendrothele asterospora***
5b. Dendrohyphidia lacking, hyphae 2.0–3 µm diam. ..... ***Xenosperma murrillii***
6a. Basidiospores lageniform ..... ***Dendrothele syspora***
6b. Basidiospores spherical or subspherical 7
7a. Surface of basidiomata with projections ..... ***Dendrothele griseocana***
7b. Surface of basidiomata smooth 8
8a. Basidiospores >15 µm diam. ..... ***Dendrothele mangiferae***
8b. Basidiospores <15 µm diam. ..... ***Dendrothele incrustans***

Descriptions

Dendrodontia bispora (Burds. & Nakasone) Guerrero & C.L.M. Rodrigues, **comb. nov.** PLATE 1

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≡ *Dendrothele bispora* Burds. & Nakasone, Mycotaxon 17: 253, 1983.

≡ *Brunneocorticium bisporum* (Burds. & Nakasone) Nakasone, Botany 87: 879, 2009.

= *Brunneocorticium pyriforme* Sheng H. Wu, Mycologia 99: 306, 2007.

BASIDIOMATA effuse, forming confluent patches 4 × 1 cm, 50–100 µm thick, closely adnate; HYMENIAL SURFACE smooth; MARGIN abrupt, pubescent, sometimes with a dark brown line along the edge.

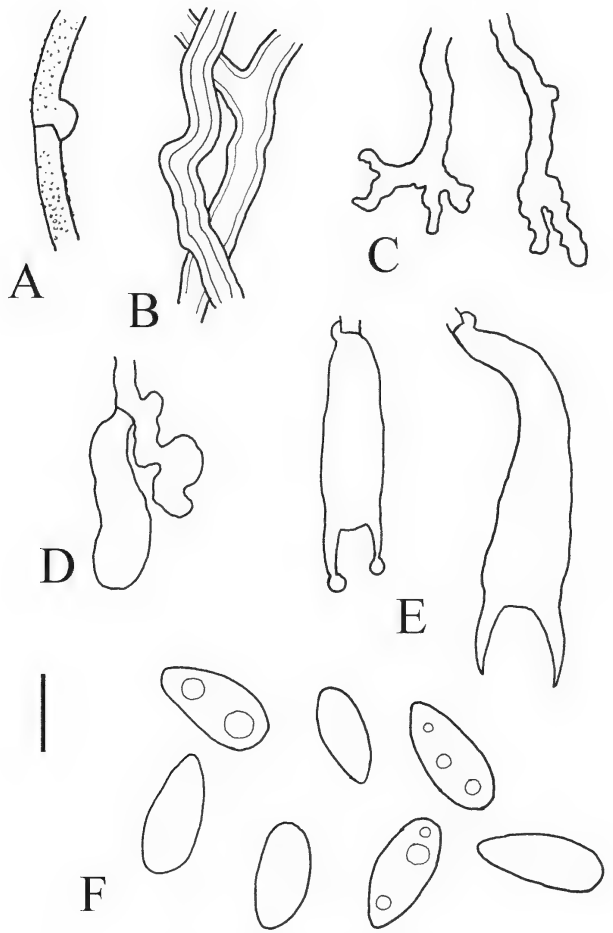

PLATE 1. *Dendrodontia bispora*.
A: generative hypha. B: skeletal hyphae. C: dendrohyphidia. D: young basidium.
E: mature basidia. F: basidiospores. Scale bar: 10 μm.

HYPHAL SYSTEM dimitic; GENERATIVE HYPHAE thin-walled, 3–4 μm in diam., finely encrusted, clamped; SKELETAL HYPHAE thick-walled, slightly brownish, sometimes flexuous and branched. CYSTIDIA lacking; DENDROHYPHIDIA branched, tortuous, 2–3 μm in diam. BASIDIA clavate, 25–40 × 7–10 μm, thin-walled, clamped at base, with 2 sterigmata, 8–10 × 2–3 μm; BASIDIOSPORES ellipsoid to ovoid, smooth, thin-walled, sometimes with droplets, 13–16.5 × 6–8 μm.

Distribution — China, Guadeloupe, Mauritius, Réunion, Taiwan, USA (Nakasone et al. 2009), and southern Brazil.

Specimens examined — **BRAZIL. Rio Grande do Sul:** Porto Alegre, Parque Farroupilha, on *Britoa guazumifolia* (*Myrtaceae*), 03.XII.1992, leg. R.T. Guerrero (ICN 97664); 12.X.2001, leg. R.T. Guerrero (ICN 102781); 31.III.2004, leg. R.T. Guerrero (ICN 102780).

Remarks — *Dendrodontia* was described based on a dimitic hyphal system with branched brownish skeletal hyphae and the presence of hyaline dendrohyphidia as main features (Hjortstam & Ryvarden 1980). Burdsall & Nakasone (1983) described *Dendrothele bispora* with a dimitic hyphal system, emphasizing its brownish skeletal hyphae as unique in the genus.

In their study of *Dendrothele*, Boidin et al. (1996) suggested excluding *D. bispora* from the genus based on its dimitic hyphal system and suggested its placement in *Dendrodontia*. Recently, Nakasone et al. (2009) transferred this species to *Brunneocorticium* Sheng H. Wu within the euagarics clade, reducing *B. pyriforme* to synonymy. Larsson (2007) and Wu et al. (2007) placed *Dendrodontia* in the polyporoid clade, but Wu et al. (2007) included only one unidentified specimen of *Dendrodontia* in their analyses.

Considering that *Dendrothele bispora* does not agree in important features with the concept of *Dendrothele* and that sequences of more specimens of *Dendrodontia* spp. (particularly of the type species *D. bicolor* (P.H.B. Talbot) Hjortstam & Ryvarden) are needed to assess the phylogenetic relationships of that genus, we prefer to transfer the species to *Dendrodontia* based on morphological evidence.

Dendrothele alliacea (Quél.) P.A. Lemke, Persoonia 3: 366, 1965. Plate 2

Basidiomata resupinate, white to ochraceous, smooth, pruinose under the lens, 120–150 µm thick, in rounded patches less than 1 cm in diam., sometimes confluent.

Hyphal system monomitic; generative hyphae irregular, 1–3 µm in diam., straight, clamps somewhat difficult to see. Leptocystidia numerous, fusiform 20–32 × 8–12 µm, slightly thick-walled ca. 1 µm thick, sometimes bearing an apical filamentous appendix; dendrohyphidia slender 1–2 µm wide bearing short branches and covered by crystals. Basidia clavate, 37–42 × 6–7 µm, tapering towards the base, clamps hardly visible, with four sterigmata, 4.5–7 µm; basidiospores ellipsoid, smooth, somewhat thick-walled, 9–13 × 5–7 µm, sometimes with granular content, with distinct apiculus, IKI–.

Distribution — Canada, South Africa, USA (Lemke 1964), Europe (Bernicchia & Gorjón 2010), Russia (Ghobad-Nejhad 2011), Uruguay (Hjortstam & Ryvarden 2007), and southern Brazil.

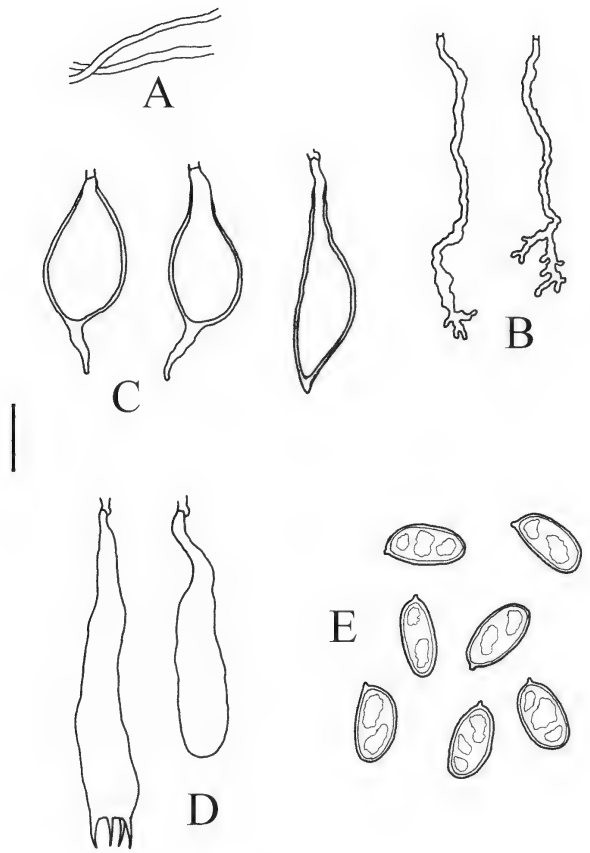

Plate 2. *Dendrothele alliacea*.
A: generative hyphae. B: dendrohyphidia. C: leptocystidia. D: mature and immature basidia. E: basidiospores. Scale bar: 10 μm.

Specimens examined — **BRAZIL. Rio Grande do Sul State:** Guaíba, Morro São Maximiano, 09.XII.2003, leg. C. Rodrigues 989 (ICN); Sapucaia do Sul, Morro Sapucaia, 18.XII.2003, leg. C. Rodrigues 1018 (ICN).

Remarks — Numerous fusiform leptocystidia are noticeable in this species. They are also found in *D. acerina* (Pers.) P.A. Lemke, from which *D. alliacea* differs by its ellipsoid spores. Our specimens have basidiospores that are a bit smaller than those mentioned by Boidin et al. (1996) but similar to the dimensions reported by Eriksson & Ryvarden (1975).

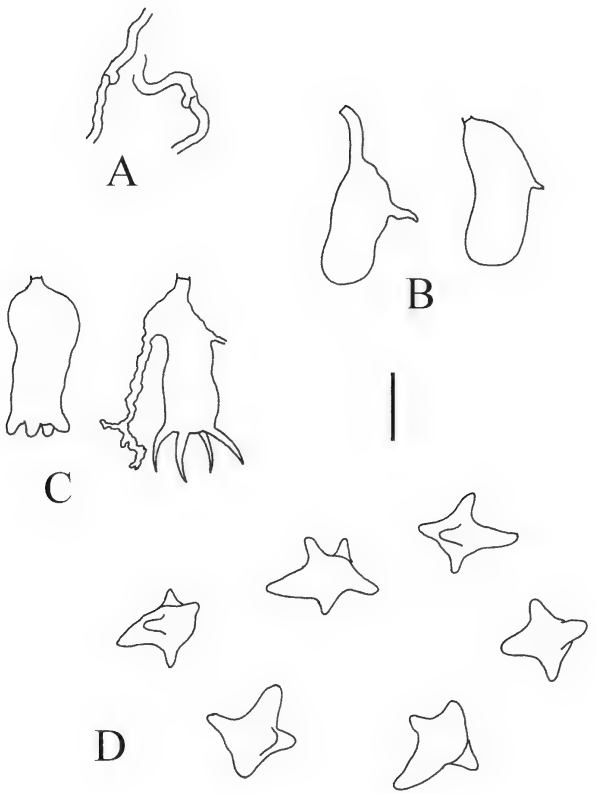

Plate 3. *Dendrothele asterospora*.
A: hyphae. B: young basidia. C: mature basidia. D: basidiospores. Scale bar: 10 µm.

Dendrothele asterospora Boidin & Lanq., Bull. Soc. Mycol. Fr. 112: 94, 1996.Plate 3

Basidiomata white to cream, in rounded patches ca. 0.5 cm wide, 40–50 µm in vertical section, covered by crystals.

Hyphal system monomitic; generative hyphae slender, tortuous, 1.5–2 µm wide, clamps difficult to see. Cystidia lacking; dendrohyphidia shortly branched. Basidia cylindric to urniform, 17–21 × 7–8 µm, frequently bearing a projection that extends as a hypha, with four sterigmata 6–7 µm long; basidiospores asteriform with 5 tips, 10–16 µm between the opposite tips, thin-walled, IKI–.

Distribution — Central African Republic, Réunion (Boidin et al. 1996, the latter sub aff.), and southern Brazil.

Specimen examined — **BRAZIL: Rio Grande do Sul State**: Sapucaia do Sul, morro Sapucaia, on *Cabralea canjerana* (*Meliaceae*), 17.IV.2003, leg. C. Rodrigues 755 (ICN).

Remarks — This species is similar to species of *Xenosperma* because of the basidia with lateral projections that resemble pleurobasidia, and pluriapiculate basidiospores, but the presence of dendrohyphidia, chalky consistency and habit points towards its placement in *Dendrothele*. This is the first report of *D. asterospora* since its original description.

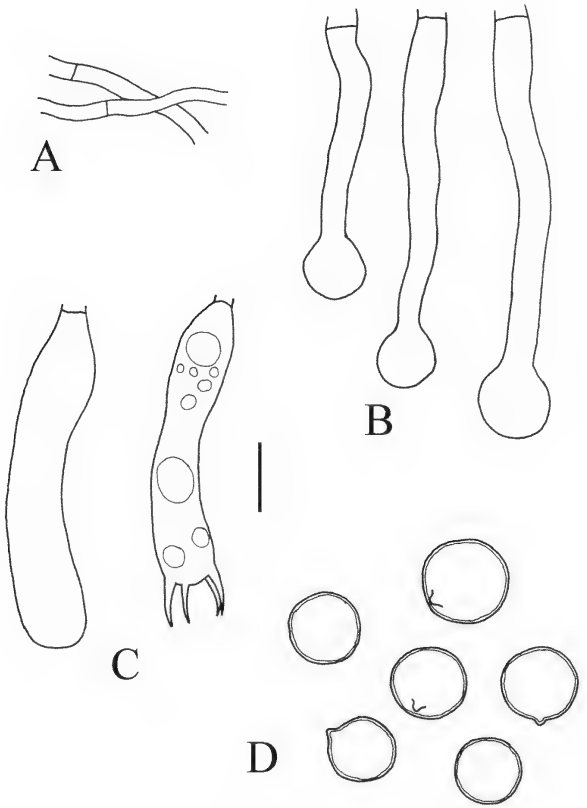

Plate 4. *Dendrothele capitulata*.
A: hyphae. B: cystidia. C: basidia. D: basidiospores. Scale bar: 10 µm.

Dendrothele capitulata Boidin & Lanq., Bull. Soc. Mycol. Fr. 112: 99, 1996. Plate 4

Basidiomata white to pale salmon, in patches, isolated or confluent, with a calcareous consistency, fragile and friable, often covered by cristals.

Hyphal system monomitic; generative hyphae 2–3 µm wide, without clamps. Cystidia lacking; dendrohyphidia irregularly branched; leptocystidia capitate, numerous, stem 3–4 µm in diam. and spherical apex 7–9 µm in diam. Basidia utriform, 40–50 × 8–10 µm, with four sterigmata,

7 µm long; BASIDIOSPORES spherical, some subspherical, 8–12.5 µm in diam., thick-walled, smooth, with a distinct apiculus, IKI–.

DISTRIBUTION — Guadeloupe and southern Brazil.

SPECIMENS EXAMINED — **BRAZIL: RIO GRANDE DO SUL STATE:** Guaíba, Morro São Maximiano, on *Luehea divaricata* (*Malvaceae*), 30.I.2003, leg. C. Rodrigues 687 (ICN); 13.V.2003, leg. C. Rodrigues 783 (ICN).

REMARKS — This species is easily recognized by its large capitate cystidia and spherical spores as described and illustrated by Boidin et al. (1996). This is the second record since its original description.

Dendrothele griseocana (Bres.) Bourdot & Galzin, Bull. Soc. Mycol. Fr. 28: 354, 1913 ["1912"]. PLATE 5

BASIDIOMATA white to ochraceous, effuse, sterile papillae visible under the lens, 40–60 µm long and distant 100 µm from each other.

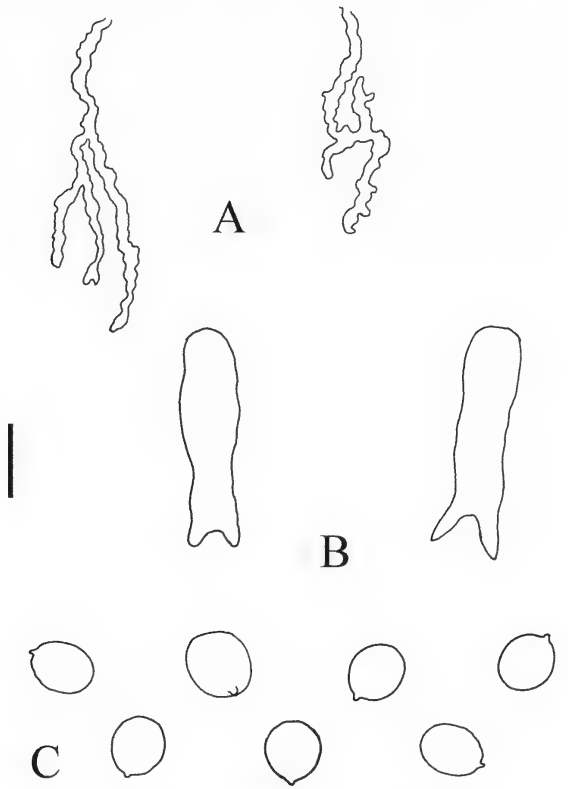

PLATE 5. *Dendrothele griseocana.*
A: dendrohyphidia. B: basidia. C: basidiospores. Scale bar: 10 µm.

HYPHAL SYSTEM monomitic; GENERATIVE HYPHAE hardly observed, tortuous, thin-walled, clamps difficult to see. CYSTIDIA lacking; DENDROHYPHIDIA numerous, richly branched, covered by crystalline matter. BASIDIA clavate when young, 25–30 × 6–7 µm with a medial constriction at maturity, with 2 sterigmata; BASIDIOSPORES ellipsoid to subspherical, smooth, slightly thick-walled, 8.5–10 × 7–9 µm, IKI–.

DISTRIBUTION — Canada, USA (Nakasone 2006), Europe (see Bernicchia & Gorjón 2011), Mexico, Uruguay (Hjortstam & Ryvarden 2007), Russia (Ghobad-Nejhad 2011), and southern Brazil.

SPECIMEN EXAMINED — **BRAZIL. RIO GRANDE DO SUL:** Guaíba, Morro São Maximiano, 09.XII.2003, leg. C. Rodrigues 1003 (ICN).

REMARKS — *Dendrothele griseocana* was traditionally considered to be a variable species. However, Nakasone (2006) resolved this species complex and characterized *D. griseocana* mainly by the presence of hyphal pegs, simple septa, and basidia with 2 sterigmata, segregated from *D. americana* Nakasone and *D. tanzaniana* Nakasone by their clamped generative hyphae and basidia with 4 sterigmata.

Dendrothele griseocana was first reported for Rio Grande do Sul State by Rick (1959).

Dendrothele incrustans (P.A. Lemke) P.A. Lemke, Persoonia 3: 366, 1965. PLATE 6

BASIDIOMATA white, formed by confluent patches 2–5 mm wide each, 50–100 µm thick; adherent, with an indistinct margin.

HYPHAL SYSTEM monomitic; SUBICULUM densely crystalline; GENERATIVE HYPHAE clamped, irregular and thin-walled, 1–2 µm in diam. CYSTIDIA lacking; DENDROHYPHIDIA 1–3 µm wide. BASIDIA clavate to suburniform, slightly swollen at the bases, 35–45 × 11–13 µm, with four sterigmata 11–13 µm long; BASIDIOSPORES spherical, sometimes with guttulae, 9–12 µm in diam., apiculus often conspicuous, IKI–.

DISTRIBUTION — Argentina, Guadeloupe, (Hjortstam & Ryvarden 2007), Canada, USA (Lemke 1964), Russia (Ghobad-Nejhad 2011), and southern Brazil.

SPECIMENS EXAMINED — **BRAZIL. RIO GRANDE DO SUL STATE:** GUAÍBA, MORRO SÃO MAXIMIANO, ON *MATAYBA ELAEAGNOIDES* (*SAPINDACEAE*), 18.III.2003, LEG. C. RODRIGUES 607 (ICN); 15.IX.2003, LEG. C. RODRIGUES 882; 15.IX.2003, LEG. C. RODRIGUES 894 (ICN).

REMARKS — The diagnostic features of *D. incrustans* are the lack of cystidia and the large spherical basidiospores with prominent apiculi. Our specimens have basidiospores slightly larger than the 8–11 µm reported by Gilbertson & Blackwell (1987) and basidia larger than 25–30 × 8–11 µm as cited by Lemke (1964).

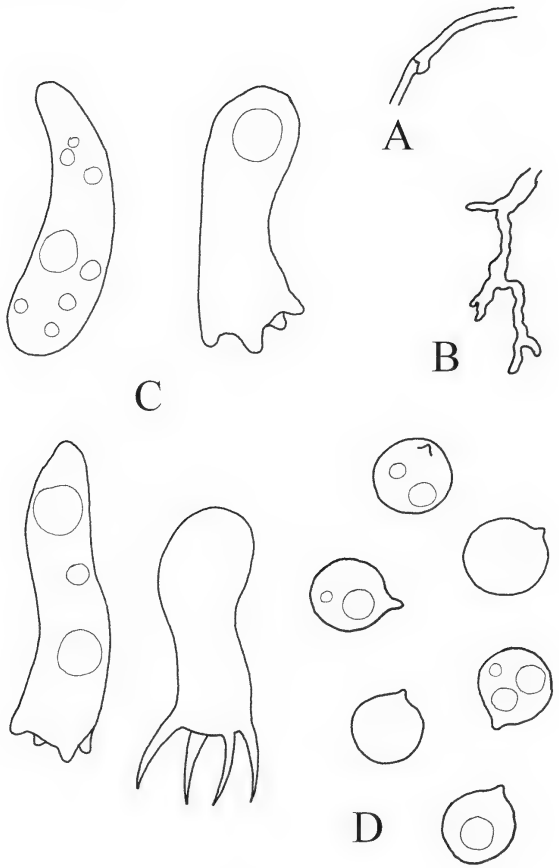

Plate 6. *Dendrothele incrustans.*
A: hypha. B: dendrohyphidium. C: basidia. D: basidiospores. Scale bar: 10 µm.

Dendrothele mangiferae Boidin & Duhem, Bull. Soc. Mycol. Fr. 112: 106, 1996.
Plate 7

Basidiomata white, occurring in irregular, rounded, small patches ca. 1 cm, 80–170 µm thick.

Hyphal system monomitic; generative hyphae 2–3 µm in diam., irregularly arranged, clamped. Dendrohyphidia thin, 1–2 µm wide, poorly branched, tips resembling a cauliflower. Basidia subcylindrical, containing oil droplets, 110–130 × 18 µm, with 45 µm long sterigmata, 25–30 × 5–6 µm, immature basidia multilayered by repetition; basidiospores spherical to subspherical 18–25 × 17–23 µm, sometimes with oil droplets and a distinctive apiculus, smooth in KOH and finely spiny in Melzer's solution, IKI–.

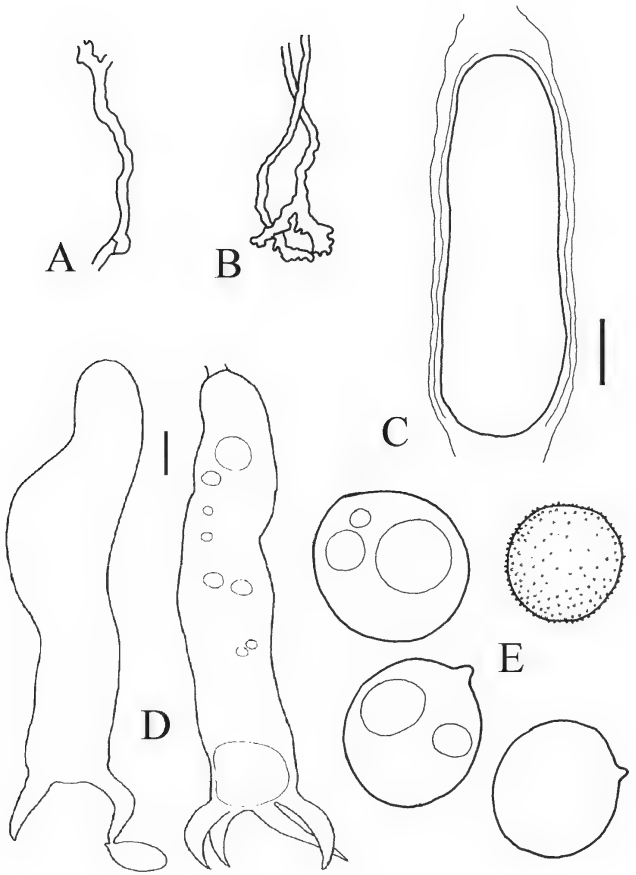

Plate 7. *Dendrothele mangiferae*.
A: hyphae. B: dendrohyphidia. C: young repetitive basidium. D: mature basidia.
E: basidiospores. Scale bars: 10 µm.

Distribution — Costa Rica (Gorjón 2012), Mauritius, Réunion (Boidin et al. 1996), and Southern Brazil.

Specimen examined — **Brazil. Rio Grande do Sul State:** Guaíba, Morro São Maximiano, on *Cedrela fissilis* (*Meliaceae*), 13.V.2003, leg. C. Rodrigues 791 (ICN).

Remarks — *Dendrothele mangiferae* has the largest basidia and basidiospores among the *Dendrothele* species discussed here. These features, which agree with the description by Boidin et al. (1996), make it easy to identify. Since its original description, *D. mangiferae* has been reported only from Costa Rica and now from southern Brazil.

Dendrothele syspora C.L.M. Rodrigues & Guerrero, **sp. nov.** PLATE 8

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Speciei Dendrothele magninavicularis *differt hyphae sine fibulae; dendrohyphida cum projecturis spinulosis; 2 sterigmatigmatis bisporatis; sporae primo conjugatis, in maturitatem secedentibunt.*

TYPE: Brazil. Rio Grande do Sul State: Viamão, Parque Estadual de Itapuã, Morro da Grota, on *Allophylus edulis* (A. St.-Hil.) Niederl. (*Sapindaceae*), 27.X.2003, leg. C. Rodrigues 924 (**Holotype,** ICN).

Etymology: *sy* = pref. Gr., together, with, joined; *spora* = L., spore.

Basidiomata in patches irregularly rounded, 3–7 mm wide, sometimes confluent, white to pale ochraceous, 100–150 µm in vertical section, texture chalky, cracking; margin indistinct, often thinning out, pruinose.

Hyphal system: monomitic; generative hyphae 2–3 µm in diam., simple-septate. Cystidia lacking; dendrohyphidia with the last branches short and with spiny projections, 1.5–3 µm in diam. Basidia: irregularly cylindrical, 34–54 × 9–12 µm, enlarged at the basis, slightly sinuous, somewhat pinched at the middle, smooth, thin-walled, with 2 sterigmata 12–12 × 3–4 µm; basidiospores navicular to lageniform, 17–24 × 7–8 µm, with a median constriction (4 × 5 µm wide), straight or curved, rarely with 1 or 2 drops, often none, smooth, thin-walled, apiculus in the broadest tip, IKI–.

Distribution — Known from three localities in Rio Grande do Sul, southern Brazil.

Additional specimens examined — **BRAZIL. Rio Grande do Sul State:** Guaíba, Morro São Maximiano, on *Ocotea puberula* (*Lauraceae*), 09.XII.2003, leg. C. Rodrigues 998 (ICN); Sapucaia do Sul, Morro Sapucaia, 18.XI.2003, leg. C. Rodrigues 1015 (ICN); leg. C. Rodrigues 1025 (ICN); leg. C. Rodrigues 1031 (ICN); Viamão, Parque Estadual de Itapuã, Morro da Grota, on *Allophylus edulis*, 11.VI.2004, leg. C. Rodrigues 1081 (ICN); leg. C. Rodrigues 1098 (ICN).

Remarks — This species presents the habit and substrate typical for *Dendrothele* species. The spores are larger than any other known species with median constriction in the genus (Nakasone & Burdsall 2011, Gorjón et al. 2011). Sterigmata and basidiospores, however, are rarely observed during their development. At first, sterigmata are born separately, but as they elongate, the tips grow toward each other and produce basidiospores that remain aligned together along their length. The spores are released together and remain joined for a short time before becoming separated. Basidiospore germination has not been observed.

Dendrothele syspora seems to belong to a recently described group of species with navicular basidiospores, including *D. cymbiformis* Nakasone & Burds., *D. magninavicularis* Nakasone & Burds., and *D. navicularis* Nakasone & Burds. from New Zealand (Nakasone & Burdsall 2011) and *D. latinavicularis* Gorjón from the Patagonian Andes forests of Argentina (Gorjón et al. 2011). *Dendrothele*

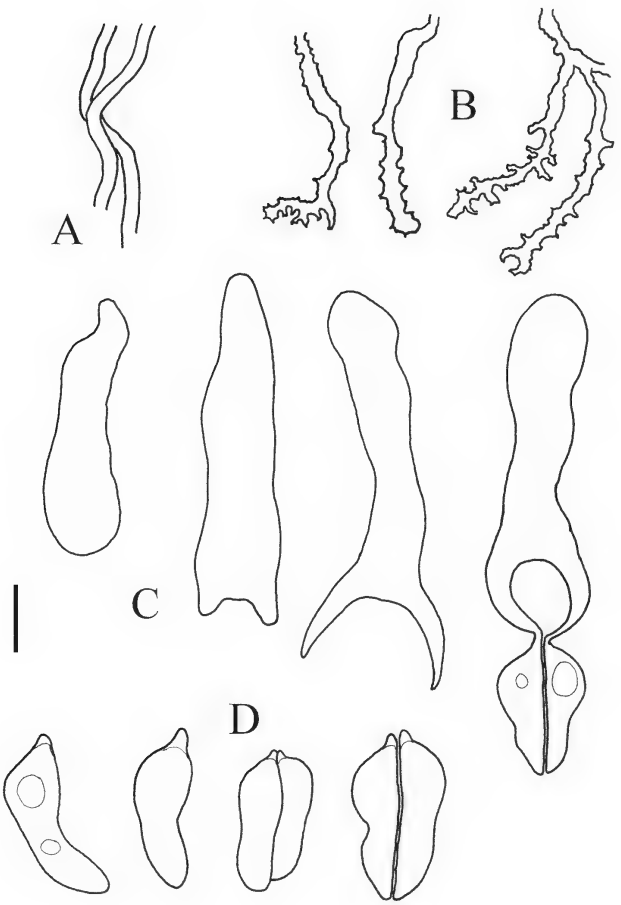

Plate 8. *Dendrothele syspora.*
A: hyphae. B: dendrohyphidia. C: basidia in different stages of development. D: basidiospores.
Scale bar: 10 μm.

latinavicularis differs from *D. syspora* by the shorter and wider basidiospores, which thus appear broadly navicular. Both *D. cymbiformis* and *D. navicularis* have smaller basidiospores, while *D. magninavicularis* basidiospores are almost the same size as those in *D. syspora*. Dendrohyphidia differ in these species: they are hyphoid in *D, cymbiformis* and *D. navicularis* but have short branches and spiny projections in *D. magninavicularis* and *D. syspora. Dendrothele syspora* is unique in this group by having simple septate generative hyphae and 2-sterigmate basidia.

Species of this group were found on different hosts: *D. latinavicularis* was always found in bark of living *Saxegothaea conspicua* Lindl., an endemic southern South American conifer (Gorjón et al. 2011); *D. cymbiformis* was reported from bark of living *Dracophyllum scoparium* Hook. f. and *D. magninavicularis* from bark of living *Melicytus ramiflorus* J.R. Forst. & G. Forst. [subsp. *ramiflorus*], both New Zealand endemic angiosperms. On the other hand, both *D. navicularis* and *D. syspora* were found in bark of several different living angiosperms native to their respective geographic regions.

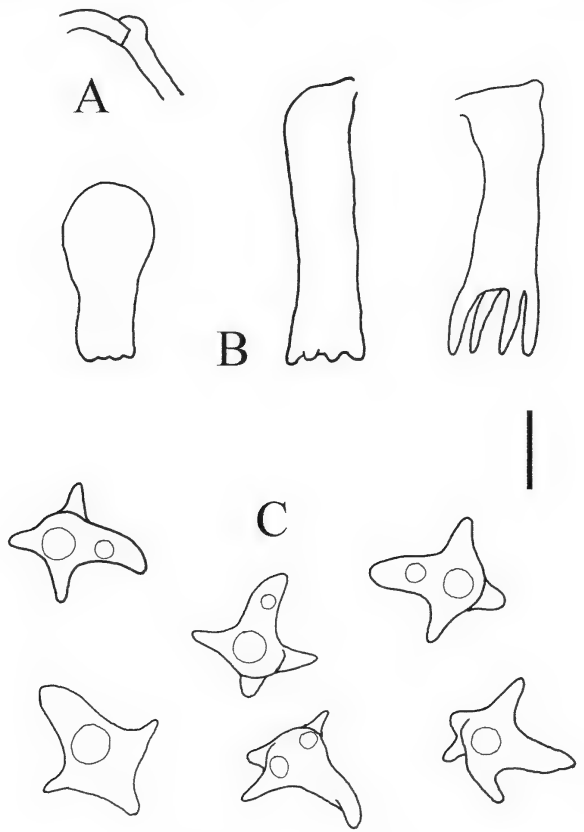

PLATE 9. *Xenosperma murrillii*.
A: hypha. B: basidia. C: basidiospores. Scale bar: 10 µm.

Xenosperma murrillii Gilb. & M. Blackw., Mycotaxon 28: 400, 1987. PLATE 9

BASIDIOMATA in patches 0.3–1.0 mm wide, sometimes confluent, white to ochraceous, 70–150 µm thick, fragile, cracking, texture chalky; MARGIN determinate, abrupt.

HYPHAL SYSTEM monomitic; GENERATIVE HYPHAE clamped, 2–3 µm in diam., thin-walled. CYSTIDIA and DENDROHYPHIDIA lacking. BASIDIA pleural, 30–40 × 9–10 µm, with a medium constriction, 4-sterigmate; BASIDIOSPORES asteriform, 17–18(–21) × 14–18 µm, with 5 projections of which one is larger than the others and curved, 1-3-gutulate.

DISTRIBUTION — USA (Gilbertson & Blackwell 1987) and southern Brazil.

SPECIMENS EXAMINED — **BRAZIL. RIO GRANDE DO SUL STATE:** Guaíba, Morro São Maximiano, on *Enterolobium contortisiliquum* (*Mimosaceae*), 15.VIII.2001, leg. C. Rodrigues 445 (ICN); 03.IX.2002, leg. C. Rodrigues 657 (ICN); Ipse, 13.V.2003, leg. C. Rodrigues 802 (ICN); Porto Alegre, Parque Farroupilha, VI.1994, leg. R. T. Guerrero (ICN 102261); on *Bauhinia forficata* (*Caesalpiniaceae*), VIII.1994, leg. R. T. Guerrero (ICN 102318); Salvador do Sul, on branch of unidentified angiosperm, 07.III.1982, leg. R. T. Guerrero (ICN 56138); Sapucaia do Sul, Morro Sapucaia, 17.IV.2003, leg. C. Rodrigues 746 (ICN); on *Allophylus edulis*, 12.VIII.2003, leg. C. Rodrigues 857 (ICN); Viamão, Parque Estadual de Itapuã, 20.X.1984, leg. R. T. Guerrero (ICN 56150).

REMARKS — *Xenosperma murrillii* differs from *X. ludibundum* (D.P. Rogers & Liberta) Oberw. ex Jülich, which has 2-sterigmate basidia and smaller basidiospores (Oberwinkler 1965). *Xenosperma pravum* Boidin & Gilles differs by having 6-sterigmate basidia (Boidin & Gilles 1989).

Discussion

Although *Dendrothele* species are frequently reported from temperate regions (Lemke 1964, Eriksson & Ryvarden 1975, Greslebin & Rajchenberg 1998) where *Corticiaceae* s.l. has been intensively studied, they also occur in tropical and subtropical regions where their diversity is poorly known. Boidin et al. (1996) described *D. asterospora*, *D. capitulata,* and *D. mangiferae* for Mauritius, Réunion, Guadeloupe, and the Central African Republic. The materials treated in this study were collected in similar climatic regions.

Other species reported here are cited both for temperate and tropical/subtropical regions. While the broad northern temperate distribution of many wood-decaying species is well established (Gilbertson 1980), more studies are needed to determine their distribution in the tropics.

Another interesting aspect of this study is the discovery of several rarely reported species occurring on substrates different from those previously cited. This includes *X. murrillii*, known only from the bark of *Juniperus virginiana* L. in the type locality. Investigation of fungi on fast-drying juniper bark, revealed that some species also grew on hardwood trees (Gilbertson & Blackwell 1987). Perhaps, host distributions are more variable in other regions where these fungi occur. The distinctive fast-drying habitat in which these fungi occur will facilitate their collection and improve their knowledge regarding distribution and host range.

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Hymenoscyphus pseudoalbidus, the correct name for *Lambertella albida* reported from Japan

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Abstract — Recent molecular analyses separate *Hymenoscyphus pseudoalbidus* (causal agent of ash dieback in Europe) from the morphologically scarcely distinguishable *H. albidus*. *Hymenoscyphus albidus* was reported (as "*Lambertella albida*") on petioles of *Fraxinus mandshurica* in Japan. Phylogenetic analysis in the present study shows Japanese "*L. albida*" to be conspecific with *H. pseudoalbidus* but with a higher genetic variability compared to European isolates. The presence of croziers at the ascus base was found to be a clear distinguishing character of *H. pseudoalbidus*. Our phylogenetic analysis of the combined ITS and LSU-D1/D2 dataset supports *Hymenoscyphus* as more appropriate than *Lambertella* for *H. pseudoalbidus*. As the *Hymenoscyphus* clade includes members with two major characters (presence of substratal stroma and brown ascospores) currently used to circumscribe *Lambertella*, the generic delimitation of *Lambertella* requires redefinition.

Key words — *Chalara fraxinea*, *Helotiaceae*, *Rutstroemiaceae*

Introduction

Ash dieback, an emerging infectious disease of common ash (*Fraxinus excelsior*), has reached epidemic levels in Central Europe during the last two decades (Bakys et al. 2009; Kowalski 2006). Since the first disease report around 1992 in Poland, ash dieback has spread into 22 European countries (Timmermann et al. 2011). The causal agent, *Chalara fraxinea* T. Kowalski was first described in Poland (Kowalski 2006). Later, its teleomorph was found and tentatively identified as *Hymenoscyphus albidus* (Gillet) W. Phillips (Kowalski & Holdenrieder 2009). Further research revealed that *H. albidus* was a complex of two species, and the ash dieback pathogen was described as a new

taxon *Hymenoscyphus pseudoalbidus* Queloz et al., separated from *H. albidus* based on differences in ITS rDNA, calmodulin gene (CAL), and translation elongation factor 1-α gene (EF1-α), as well as substantial differences in inter-simple sequence repeat anchored PCR (ISSR-PCR) fingerprinting (Queloz et al. 2011). The genetic distinction between *H. albidus* and *H. pseudoalbidus* was later confirmed by population genetic studies (Bengtsson et al. 2012, Gross et al. 2011, Husson et al. 2011). Queloz et al. (2011) indicated that the two species are indistinguishable in apothecial morphology except for a tendency toward slightly longer ascospores in *H. pseudoalbidus*. In fact, measurements in the two species overlapped, making it virtually impossible to distinguish *H. pseudoalbidus* from *H. albidus* based on ascospore size (Queloz et al. 2011).

However, recent morphological studies of European materials revealed a sharp difference in the ascus base that permits unequivocal distinction between *H. albidus* and *H. pseudoalbidus* (Baral & Bemmann, in prep.). The importance of ascal croziers in helotialean fungi was discovered by White (1942, 1943, 1944). Although many later workers neglected this discovery, a number of studies have confirmed that the presence or absence of ascal croziers is a stable characteristic that clearly distinguishes between closely allied species (e.g., Baral 1984; Huhtinen 1989).

Carpenter (1981) transferred *Hymenoscyphus albidus* to *Lanzia* based on the presence of a conspicuous substratal stroma. The transfer to *Lambertella* (Korf 1982) was based on the presence of some brown spores in the studied specimen (R.P. Korf, pers. comm.), and this recombination was confirmed when Japanese material was studied by Hosoya et al. (1993), who likewise found the ascospores to become brown prior to germination. Although these two characters were also recognized in the teleomorph classification of *Chalara fraxinea*, Kowalski & Holdenrieder (2009), who followed European usage to classify the species in *Hymenoscyphus,* did not place the species in *Lambertella.*

Lambertella albida (Gillet) Korf was reported as a new record for Japan by Hosoya et al. (1993) from petioles of *Fraxinus mandshurica*, a common ash in northeastern Asia. The asexual reproductive structure was also obtained in pure culture of Japanese material, but the authors regarded it as having a spermatial function. Since conidiophores have never been reported for *H. albidus*, and *H. pseudoalbidus* was considered morphologically indistinguishable from *H. albidus*, the taxonomic status of "*L. albida*" as reported from Japan needs to be reconsidered.

The aims of the present research were: 1) to clarify whether Japanese "*L. albida*" belongs to *H. albidus* or *H. pseudoalbidus* based on molecular phylogeny; 2) to examine if the presence of croziers is appropriate to separate the two species; 3) to clarify whether *Hymenoscyphus* or *Lambertella* is more appropriate for Japanese specimens currently known as "*L. albida*".

Materials & methods

Specimens and strains

Thirty-one specimens were analyzed, which include nine "*L. albida*" specimens from Japan, sixteen further specimens classified as *Lambertella* spp., four specimens classified as *Hymenoscyphus* spp., and two *Lachnum* species as outgroup (Table 1). Japanese isolates were obtained from single spores, using a Skerman's micromanipulator (Skerman 1968). Three *L. albida*" isolates of (FC-1445, FC-2793, FC-2799) were maintained on potato dextrose agar (PDA, Nissui, Tokyo, Japan) slants and deposited in Biological Resource Center, National Institute of Technology and Evaluation (NITE-BRC, Japan).

Observation of ascal base and anamorph

The bases of asci were examined for the presence of croziers or simple septa in Japanese specimens previously identified as "*L. albida*". Apothecial fragments were mounted in either water + 1% phloxine B solution or 5–10% KOH + Congo Red and strongly squashed.

In order to observe the anamorph, isolates were incubated on malt extract agar (MEA, 20 g malt extract, 20 g agar, 1000 ml distilled water) at room temperature up to 1 month.

DNA extraction, PCR amplification and sequencing

Isolates were cultivated in 2 ml of 2% malt extract broth (20 g malt extract, 1000 ml distilled water) for 2 weeks to obtain mycelium. Genomic DNA of isolates was obtained from about 50 mg of frozen mycelia using a DNeasy Plant Mini Kit (Qiagen, Mississauga, ON, Canada) following the manufacturer's instruction. Genomic DNA from apothecia was extracted using the modified Cetyltrimethylammonium bromide (CTAB) extraction following glass milk purification methods as summarized by Hosaka (2009) and Hosaka & Castellano (2008). Briefly, samples were ground in liquid nitrogen using mortar and pestle, incubated in CTAB buffer (2% CTAB, 100 mM Tris pH 8.0, 20 mM EDTA, 1.4 M NaCl) at 65°C for 1 hour, and proteins were removed using the mixture of chloroform: isoamylalcohol (24: 1). The materials were further purified using 6M sodium iodine buffer (1M Tris pH 6.8, 2M Na_2SO_3) with glass milk, washed with ethanol/buffer solution (10 mM Tris pH 7.4, 1mM EDTA, 100 mM NaCl, 50% EtOH), and finally eluted in 100 µl Tris-EDTA buffer (TE, 10 mM Tris-HCl pH 8.0, 1 mM EDTA).

The internal transcribed spacer regions (ITS-5.8S), the LSU-D1/D2 region of the large subunit rDNA gene (28S rDNA), the calmodulin gene (CAL) and translation elongation factor 1- α (EF1-α) were sequenced using primer pairs ITS1F and ITS4 or ITS1 and ITS4 (White et al. 1990), NL1 and NL4 (O'Donnell 1993), Cal-228F and Cal-737R (Carbone & Kohn 1999), EF1-728F and EF1α_R (Carbone & Kohn 1999, Grünig et al. 2007), respectively. PCR reactions were performed using 10 µl reaction volumes each containing: 0.5 µl genomic DNA, 0.25 µl of each primer (10 µM), 0.05 µl (0.25 unit) of ExTaq DNA polymerase (TaKaRa, Tokyo, Japan), 5.0 µl deoxynucleotide triphosphate (dNTP) mixture containing 2.5 mM each dNTP and ExTaq buffer containing 2 mM Mg^{2+} (adding 3.95 µl distilled water to get 10 µl reaction volumes). Amplifications were performed for ITS-5.8S and D1/D2 region with preliminary denaturation at 94°C for

3 min, 35 amplification cycles (94°C for 1 min, 52°C for 30 s and 72°C for 1 min), followed by a final extension at 72°C for 7 min. For both CAL and EF1-α regions, the annealing temperature was set at 54°C for 30s. PCR products were electrophoresed in 1% agarose gels stained with ethidium bromide and visualized under UV light. PCR products were then purified using an ExoSAP-IT purification kit (USB, Cleveland, OH, USA) following the manufacturer's instruction.

Sequencing was carried out using the Big Dye Terminator Cycle Sequencing Kit on the DNA auto sequencer 3130x (Applied Biosystems Inc., Norwalk, CT, USA), following the manufacturer's instructions. Sequences were assembled and edited by SeqMan (Lasergen v6 DNAstar), and congruent sequences obtained from both strands were saved. A total of 92 sequences generated from this study were deposited in GenBank (Table 1).

Phylogenetic analyses

The obtained DNA sequences were aligned by Clustal W using the default parameters (Thompson et al. 1994) and edited manually when necessary using BioEdit ver. 7.0.5.2 (Hall 1999). Ambiguous regions having gaps were excluded from the analyses by checking the alignment in BioEdit. Each of the three datasets (ITS-5.8S, CAL and EF1-α) including all sequences of European *H. albidus* and *H. pseudoalbidus* used in the analyses of Queloz et al. (2011) and Japanese "*L. albida*" species were analyzed, rooted with other *Hymenoscyphus* species as used in the analyses of Queloz et al. (2011) (*H. scutula*, *H. fructigenus*, *H. caudatus*, *H. serotinus* for ITS-5.8S; *H. fructigenus* for CAL and EF1-α). 103 sequences downloaded from GenBank shown in Fig. 1–3 were used. A combined sequence data set (ITS-5.8S and D1/D2 region) was also analyzed, including seven "*L. albida*", 24 *Lambertella* species, nine *Hymenoscyphus* species, and rooted with *Lachnum abnorme* and *Lachnum virgineum*.

Maximum parsimony (MP) and maximum likelihood (ML) methods were used to analyze all four datasets. The combination of these two methods was suggested as a supportive way when constructing a phylogenetic tree (Kolaczkowski & Thornton 2004). The MP analyses were performed using PAUP version 4.0b10 (Swofford 2002). Heuristic searches were conducted with tree bisection–reconnection branch swapping algorithm (TBR), random sequence additions, and with Multrees option on. Bootstrap values (BP) of the most parsimonious trees were obtained from 1000 replications.

The ML analyses were conducted using GARLI version 0.951 (Zwickl 2006). The analyses were conducted using the GTR+G+I model (six general time reversible substitution rates, assuming gamma rate heterogeneity and a proportion of invariable sites), with model parameters estimated over the duration of specified runs. The tree topology with the highest likelihood was inferred from 10 independent runs from random starting trees. The "stopgen" parameters were set to 50,000,000 and other parameters were set to default values. Bootstrap analyses were done using 1000 replications with the same parameters as the initial tree search. The data matrix and trees have been deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study /TB2:S12500).

Genetic variation analysis

DnaSP Version 4.0 software (Librado & Rozas 2009) was used to investigate the genetic diversity among the European and Japanese populations of *H. pseudoalbidus*.

TABLE 1 Specimens, pure cultures, and sequences used in the present study.

SPECIES	PURE CULTURE	SPECIMEN	LOCALITY	SUBSTRATE	GENBANK ACCESSION NO.			
					ITS	D1-D2	CAL	EF1-α
Hymenoscyphus pseudoalbidus (as "*Lambertella albida*")	NA	TNS-F-52061	Yubari, Hokkaido Pref.	Petiole of *Fraxinus mandshurica*	AB705222	AB705262	—	—
	NA	TNS-F-52060	Tomakomai-shi, Hokkaido Pref.		—	—	—	—
	NA	TNS-F-52062	Ueda-shi, Nagano Pref.		—	—	—	—
	NA	TNS-F-12503	Sugadaira, Nagano Pref.		AB705221	AB705261	AB705208	AB705214
	FC-1445	TNS-F-12761	Sugadaira, Nagano Pref.		AB705218	AB705258	AB705209	AB705215
	FC-2793	TNS-F-40043	Yubari, Hokkaido Pref.		AB705219	AB705259	AB705206	AB705212
	FC-2799	TNS-F-40051	Chitose, Hokkaido Pref.		AB705220	AB705260	AB705207	AB705213
	NA	TNS-F-40074	Sugadaira, Nagano Pref.		AB705223	AB705263	AB705210	AB705216
	NA	TNS-F-17817	Sugadaira, Nagano Pref.		AB705224	AB705264	AB705211	AB705217
Hymenoscyphus fructigenus (Bull.) Gray	FC-2375	TNS-F-31299	Ushiku-shi, Ibaraki Pref.	Acorn	AB705225	AB705265	—	—
	FC-1093	TNS-F-11208	Towada-ko-machi, Aomori Pref.	Leaf of *Ginkgo biloba*	AB705226	AB705270	—	—
Hymenoscyphus ginkgonis J.G. Han & H.D. Shin	FC-1491	NA	—	—	AB705227	AB705266	—	—
	FC-1492	NA	—	—	AB705228	AB705268	—	—
	FC-1493	NA	—	—	AB705229	AB705269	—	—
	FC-1494	NA	—	—	AB705230	AB705267	—	—
Hymenoscyphus herbarum (Pers.) Dennis	NBRC-9520	NA	—	—	AB705231	AB705271	—	—
Hymenoscyphus. sp. 1	FC-2727	TNS-F-40037	Ueda-shi, Nagano Pref.	Grass stem	AB705232	AB705272	—	—
Hymenoscyphus sp. 2	FC-2725	TNS-F-40029	Ueda-shi, Nagano Pref.	Unknown leaf	AB705233	AB705273	—	—
Lachnum abnorme (Mont.) J.H. Haines & Dumont	FC-2118	TNS-F-16609	Ashigarakami-gun, Kanagawa Pref.	Wood of *Cephalotaxus harringtonia*	AB705234	AB705274	—	—
Lachnum virgineum (Batsch) P. Karst.	FC-2137	TNS-F-16583	Ashigarakami-gun, Kanagawa Pref.	Unknown	AB705235	AB705275	—	—
Lambertella advenula (W. Phillips) Hosoya & Y. Otani	FC-1007	TNS-F-11194	Agatsuma, Gunma Pref.	Needle of *Larix* sp.	AB705236	AB705276	—	—
	FC-2718	TNS-F-40015	Ueda-shi, Nagano Pref.		AB705237	AB705277	—	—
	FC-2722	TNS-F-40019	Ueda-shi, Nagano Pref.		AB705238	AB705278	—	—

Table 1 Continued

Species	Pure culture	Specimen	Locality	Substrate	GenBank accession no.			
					ITS	D1-D2	CAL	EF1-α
Lambertella cornimaris Höhn.	FC-2389	TNS-F-30402	Bunkyo-ku, Tokyo	Leaf of *Mallotus japonicus*	AB705239	AB705279	—	—
Lambertella sp. 1	NBRC- 9067	NA	—	—	AB705240	AB705280	—	—
	FC-1487	NA	—	—	AB705241	AB705281	—	—
	FC-1488	NA	—	—	AB705242	AB705282	—	—
	FC-1489	NA	—	—	AB705243	AB705283	—	—
	FC-1490	NA	—	—	AB705244	AB705284	—	—
	FC-2645	TNS-F-36994	Chiyoda-ku, Tokyo	Petiole of *Fatsia japonica*	AB705245	AB705287	—	—
	FC-1985	TNS-F-30018	Chiyoda-ku, Tokyo		AB705247	AB705286	—	—
	FC-1190	TNS-F-31157	Odawara-shi, Kanagawa Pref.		AB705246	AB705285	—	—
	NBRC- 6894	NA	-	-	AB705248	AB705288	—	—
Lambertella sp. 2	FC-1230	TNS-F-17633	Kikuchi-shi, Kumamoto Pref.	Leaf of *Aucuba japonica*	AB705249	AB705289	—	—
Lambertella sp. 3	FC-2726	TNS-F-40033	Ueda-shi, Nagano Pref.	Leaf of *Pinus* sp.	AB705250	AB705290	—	—
Lambertella sp. 4	NA	TNS-F-40036	Ueda-shi, Nagano Pref.	Unknown leaf	AB705251	AB705291	—	—
Lambertella sp. 5	NA	TNS-F-40038	Ueda-shi, Nagano Pref.	Unknown leaf	AB705252	AB705292	—	—
Lambertella sp. 6	NA	TNS-F-40026	Iriomote Isl., Okinawa Pref.	Unknown leaf	AB705253	AB705293	—	—
Lambertella sp. 7	NA	TNS-F-40027	Tsukuba-shi, Ibaraki Pref.	Leaf of *Camellia japonica*	AB705254	AB705294	—	—
Lambertella sp. 8	NA	TNS-F-40031	Ueda-shi, Nagano Pref.	Leaf of *Pinus* sp.	AB705255	AB705295	—	—
Lambertella yunnanensis (S.H. Ou) W.Y. Zhuang & Yan H. Zhang	NA	TNS-F-40035	Ueda-shi, Nagano Pref.	Branch of *Pinus* sp.	AB705256	AB705296	—	—
	NA	TNS-F-40028			AB705257	AB705297	—	—

* NA: not available. Apothecia were used for DNA extraction when isolates not present.

The alignments of the ITS region, and the portion of CAL and EF1-α regions were separately compared without deletions, and the genetic diversity parameter Pi was calculated.

Results

Taxonomic reconsideration of Japanese "*Lambertella albida*" based on three loci

The aligned ITS-5.8S rDNA dataset comprised 454 bp, and 11 ambiguously aligned sites (site nos. 100–102, 336–343) were excluded from the analyses. Three most parsimonious trees were obtained [tree length (TL): 67 steps, consistency index (CI): 0.8060, retention index (RI): 0.9615, rescaled consistency index (RC): 0.7750]; one is shown in Fig. 1. The ML analysis yielded an optimal tree with the best likelihood (log likelihood = –1051.7137), topologically congruent with this MP tree, which highly supported monophyly of the ingroup. In the ingroup, two major clades were supported with high BP (BP >80%) confirming Queloz et al. (2011); Japanese materials were included in the strongly supported (92/90% ML BP/MP BP) clade with European *H. pseudoalbidus*.

The aligned CAL dataset comprised 460 bp, and 18 ambiguously aligned sites (site nos. 38–42, 72–74, 82, 176–177, 201, 216–217, 247–250) were excluded from the analyses. One of the 10 MP trees obtained (TL: 28 steps, CI: 0.9286, RI: 0.9888, RC: 0.9182) is shown in Fig. 2. The ML tree (log likelihood = –1110.7132) showed the same topology with one MP tree. Here also two major clades were highly supported and Japanese materials were included in the European *H. pseudoalbidus* clade with high support.

The aligned EF1-α dataset comprised 553 bp with 31 ambiguously aligned sites (site nos. 35–36, 86–87, 121–127, 221–228, 233–239, 453–456) excluded from further analysis. Only one parsimonious tree (Fig. 3) was obtained (TL: 28 steps, CI: 0.8929, RI: 0.9779, RC: 0.8732). The ML tree (log likelihood = –1145.5894) did not show any topological conflict with the MP tree. Also, two major clades were obtained, one consisting of European *H. albidus* with high support and the other (including Japanese "*L. albida*" and *H. pseudoalbidus*) that was moderately supported (61/–%). In the latter clade, five out of six Japanese "*L. albida*" materials grouped with European *H. pseudoalbidus* with high support (97/97%).

Morphology of Japanese "*Lambertella albida*"

The presence of croziers at the base of asci was observed in all nine Japanese "*L. albida*" specimens (Fig. 4). White (1944) observed the absence of croziers in the type of *H. albidus;* Baral (Baral & Bemmann,in prep.) observed the presence of croziers in the type of *H. pseudoalbidus,* and he and Bemmann examined numerous European materials (including some specimens treated by Queloz et al. 2011) morphologically. The presence of basal croziers was found to be a clear distinguishing character between the two taxa (Baral & Bemmann,in prep.).

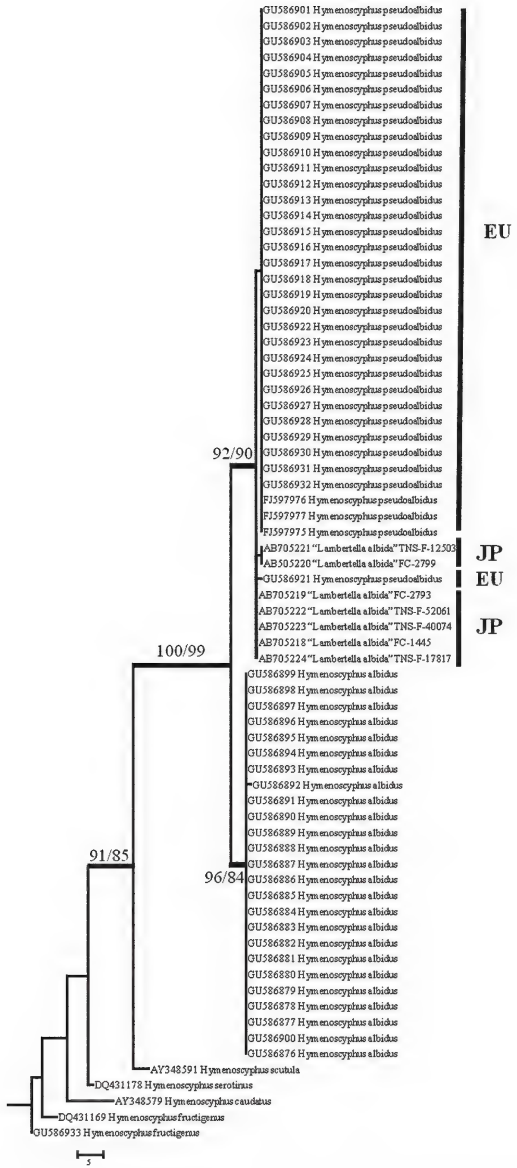

FIG. 1. One of three most parsimonious trees based on ITS rDNA sequences from Japanese "*Lambertella albida*" and European *Hymenoscyphus albidus* and *H. pseudoalbidus*. Numbers above the branches are maximum parsimony (MP) bootstrap values (BP) followed by maximum likelihood (ML) BP >50% in 1000 replications. Tree length: 67 steps; consistency index: 0.8060; retention index: 0.9615; rescaled consistency index: 0.7750. EU: European materials; JP: Japanese materials

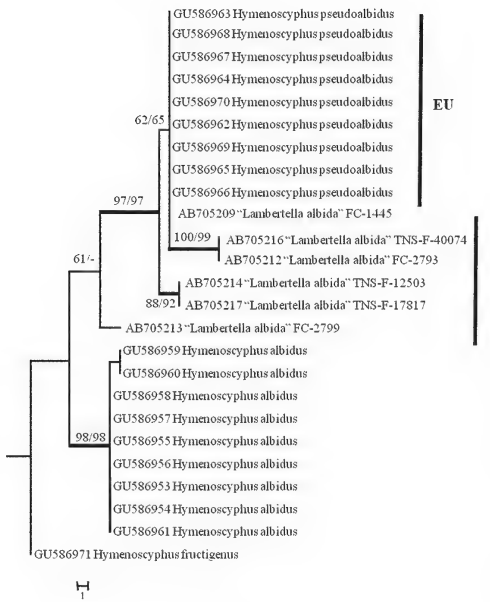

FIG. 2. One of ten most parsimonious trees based on CAL gene sequence data of Japanese "*Lambertella albida*" with European *Hymenoscyphus albidus* and *H. pseudoalbidus*. Numbers above the branches are bootstrap values (BP) in maximum parsimony analysis (MP) followed by BS from maximum likelihood (ML) >50% in 1000 replications. Tree length: 28 steps; consistency index: 0.9286; retention index: 0.9888; rescaled consistency index: 0.9182. EU: European materials; JP: Japanese materials

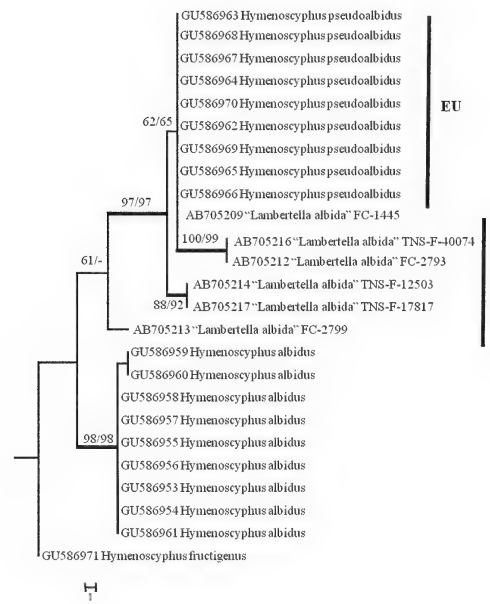

FIG. 3. Maximum parsimony tree based on EF1-α gene sequences from Japanese "*Lambertella albida*" and European *Hymenoscyphus albidus* and *H. pseudoalbidus*. Numbers above the branches are maximum parsimony (MP) bootstrap values (BP) followed by maximum likelihood (ML) BP >50% in 1000 replications. Tree length: 28 steps; consistency index: 0.8929; retention index: 0.9779; rescaled consistency index: 0.8732. "–": BP ≤50%; EU: European materials; JP: Japanese materials

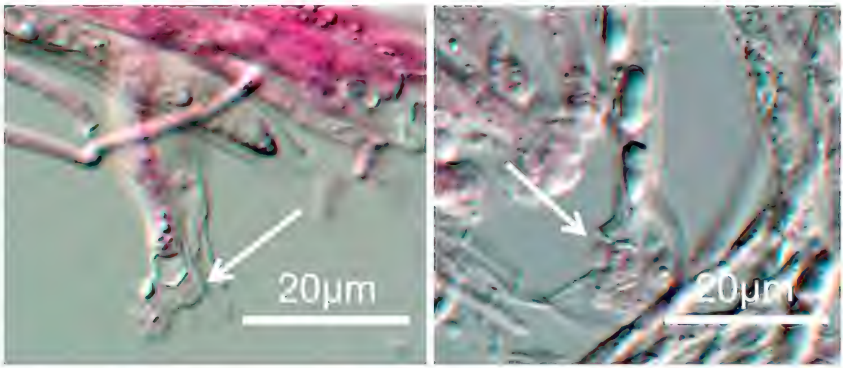

Fig. 4. Representative photos of croziers at the base of asci observed in Japanese materials of *Hymenoscyphus pseudoalbidus*. Mounted in 1% phloxine B solution (A. TNS-F-12503; B. TNS-F-52061). Scale = 20 µm

The conidia and conidiophores of *Chalara fraxinea* were obtained on MEA, but conidia did not germinate in any of the isolates during the prolonged incubation up to one month at room temperature.

Results from the above molecular, morphological, and cultural analyses all indicate that Japanese "*L. albida*" materials should be re-identified as *H. pseudoalbidus*.

Comparison of genetic variation between Japanese and European materials

Japanese *H. pseudoalbidus* showed a higher genetic variation compared with European *H. pseudoalbidus* and *H. albidus* in all the three genes, whereas no genetic difference was observed within European *H. pseudoalbidus* for CAL and EF1-α genes, and within European *H. albidus* for CAL gene. (Table 2).

Comparison of nucleotide characters between Japanese and European materials

Nucleotide differences in the EF1-α, CAL and ITS genes were found between European *H. pseudoalbidus* and *H. albidus* (Queloz et al. l.c.). Although most nucleotides from Japanese *H. pseudoalbidus* populations matched those from European *H. pseudoalbidus* populations, some positions deviated. Unexpectedly, these deviating positions consistently showed the same nucleotides as *H. albidus*. EF1-α gene nucleotides were most diverse among Japanese *H. pseudoalbidus* materials (Table 3). When comparing the 22 positions in which European *H. pseudoalbidus* differed from *H. albidus*, Japanese material showed concordance with *H. pseudoalbidus* in 7 positions, while the remaining 15 positions showed variation within Japanese materials. Thereby, each position that differed from European *H. pseudoalbidus* showed the nucleotide that characterized *H. albidus*. This phenomenon occurred mainly in positions included in the definition (protologue) of *H. pseudoalbidus* (Queloz et al. 2011), but also in 4 additional positions (232-235 in Table 3) that were not included

Table 2. Nucleotide diversity (Pi) between strains of European *H. pseudoalbidus*, Japanese *H. pseudoalbidus* ("*Lambertella albida*"), and European *H. albidus*.

Loci	Nucleotide diversity (Pi)		
	European HP	Japanese HP	European HA
ITS	0.00034 (n=35)	0.00287 (n=7)	0.00096 (n=25)
CAL	0.00000 (n=9)	0.01147 (n=6)	0.00000 (n=9)
EF1-α	0.00000 (n=9)	0.02188 (n=6)	0.00071 (n=7)

Table 3. EF1-α gene nucleotide comparisons in European *H. pseudoalbidus*, Japanese *H. pseudoalbidus* ("*Lambertella albida*"), and European *H. albidus*.

	Definition	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N
	Position	40	67	89	127	143	148	180	189	192	199	211	215	223	230	236	241	260	453	232	233	234	235
	EU HP	A	T	G	C	T	C	C	T	A	C	G	T	T	C	G	G	A	A	C	C	A	T
	FC-1445	A	T	G	C	T	C	C	T	A	C	G	T	T	C	G	G	A	A	C	C	A	T
	TNS-F-40074	A	T	G	C	T	C	C	T	A	C	G	T	T	C	G	G	A	G	C	C	A	T
JP	FC-2793	A	T	G	C	T	C	C	T	A	C	G	T	T	C	G	G	A	G	C	C	A	T
HP	TNS-F-12503	A	T	G	C	T	C	C	C	A	C	G	T	T	C	G	G	A	A	T	C	A	T
	TNS-F-17817	A	T	G	C	T	C	C	C	A	C	G	T	T	C	G	G	A	A	T	C	A	T
	FC-2799	A	T	G	C	T	C	A	C	C	T	G	C	C	A	GAP	C	G	A	T	T	GAP	GAP
	EU HA	G	C	A	G	C	G	A	C	C	T	A	C	C	A	GAP	C	G	G	T	T	GAP	GAP

* Y: Positions included in the definition of *H. pseudoalbidus* (protologue); N: Out of definition; EU: European; JP: Japanese; HP: *H. pseudoalbidus*; HA: *H. albidus*.

in the protologue. One strain (FC-2799) shared 14 nucleotides with *H. albidus* and 8 with *H. pseudoalbidus*, suggesting it is intermediate between these two species (Fig. 3, Table 3).

However, strain FC-2799 and four other Japanese materials concurred with *H. pseudoalbidus* in all 21 CAL gene positions where the European *H. pseudoalbidus* differed from *H. albidus*. Within these positions, some Japanese specimens showed variation at position 244 (specimens: TNS-F-40043, TNS-F-12503) and position 266 (specimen: TNS-F-12503) where they shared, instead, nucleotides with *H. albidus*. Further CAL region variation was noted at positions where European *H. albidus* and *H. pseudoalbidus* do not differ.

In the ITS rDNA, Japanese materials concurred with European *H. pseudoalbidus* except for two positions (site nos. 83, 124) at which all Japanese materials showed the character of *H. albidus*.

Generic taxonomy of *Hymenoscyphus pseudoalbidus*

The combined ITS-5.8S and D1/D2 dataset comprised 1183 bp (582 bp for ITS-5.8S rDNA, 601 bp for D1/D2), and 15 ambiguously aligned ITS sites (site nos. 80–81, 107–113, 127, 140–141, 206, 581–582) and 17 ambiguously aligned D1/D2 sites (site nos. 583–596, 781–782, 850) were excluded from the analysis. One of the 16406 equally parsimonious trees obtained (TL: 67 steps, CI: 0.8060, RI: 0.9615, RC: 0.7750) is shown in Fig. 5. The ML tree (log likelihood = –6682.1630) did not show any topological conflict with the MP trees. In the MP consensus tree and the best ML tree, two major clades were generated, one strongly supported (100/100%) and the other moderately supported (77/80%). The more strongly supported clade was composed solely of *Lambertella* species, including the type species *L. corni-maris*. The other clade included the *Hymenoscyphus* species and *L. yunnanensis*; *H. pseudoalbidus* grouped here with *H. fructigenus* (type species of *Hymenoscyphus*) with 99% BP support. This phylogenetic analysis shows *H. pseudoalbidus* as more distantly related to the *Lambertella* core group (including the type) and more closely related to the *Hymenoscyphus* core group (including the type).

Discussion

Based on the rapid expansion and high intensity of ash dieback in Europe, *H. pseudoalbidus* was considered as an invasive alien organism (Husson et al. 2011; Queloz et al. 2011; Timmermann et al. 2011). However, until now its

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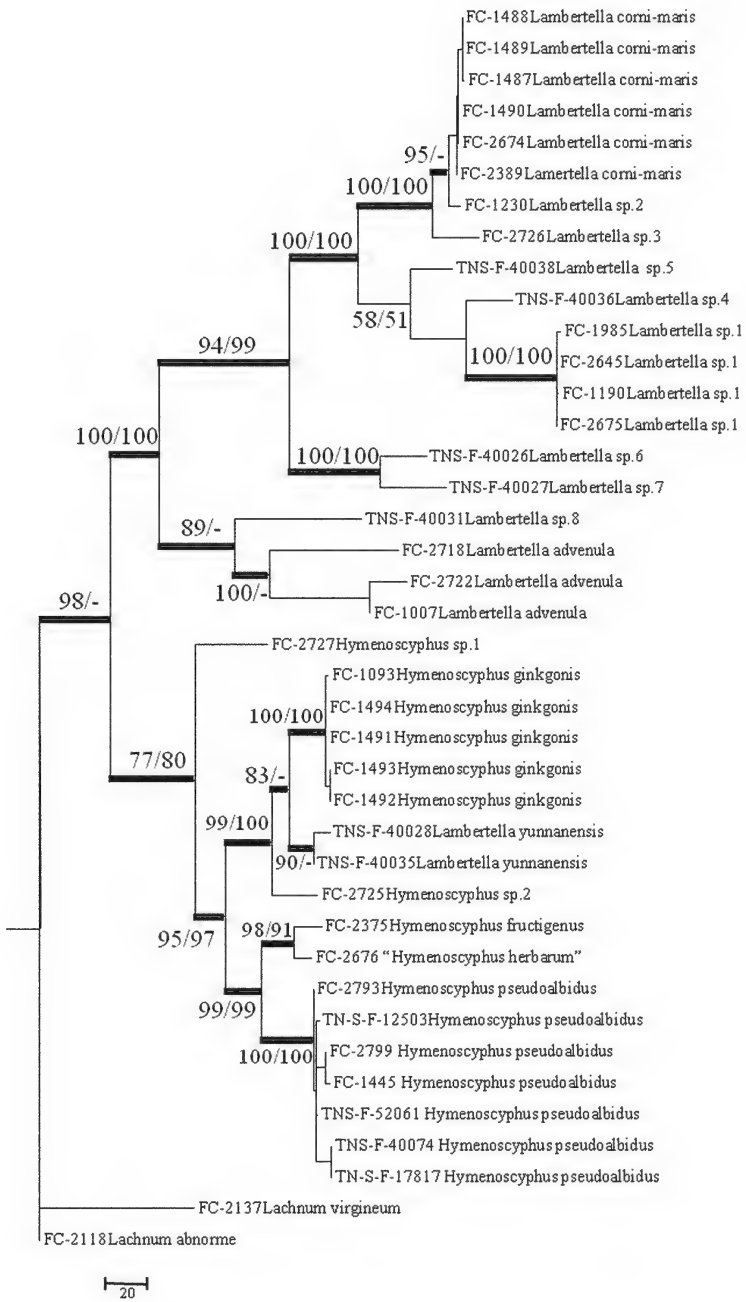
Fig. 5. One of 16406 most parsimonious trees inferred from combined dataset of ITS and D1-D2 sequences showing the relationship of Japanese *Hymenoscyphus pseudoalbidus* with other *Hymenoscyphus** and *Lambertella* species. Numbers above the branches are maximum parsimony (MP) bootstrap values (BP) followed by maximum likelihood (ML) BP >50% in 1000 replications. Tree length: 67 steps; consistency index: 0.8060; retention index: 0.9615; rescaled consistency index: 0.7750. "–": BP ≤50%.


FC-1488Lambertella corni-maris
FC-1489Lambertella corni-maris
FC-1487Lambertella corni-maris
FC-1490Lambertella corni-maris
FC-2674Lambertella corni-maris
FC-2389Lamertella corni-maris
FC-1230Lambertella sp.2
FC-2726Lambertella sp.3
TNS-F-40038Lambertella sp.5
TNS-F-40036Lambertella sp.4
FC-1985Lambertella sp.1
FC-2645Lambertella sp.1
FC-1190Lambertella sp.1
FC-2675Lambertella sp.1
TNS-F-40026Lambertella sp.6
TNS-F-40027Lambertella sp.7
TNS-F-40031Lambertella sp.8
FC-2718Lambertella advenula
FC-2722Lambertella advenula
FC-1007Lambertella advenula
FC-2727Hymenoscyphus sp.1
FC-1093Hymenoscyphus ginkgonis
FC-1494Hymenoscyphus ginkgonis
FC-1491Hymenoscyphus ginkgonis
FC-1493Hymenoscyphus ginkgonis
FC-1492Hymenoscyphus ginkgonis
TNS-F-40028Lambertella yunnanensis
TNS-F-40035Lambertella yunnanensis
FC-2725Hymenoscyphus sp.2
FC-2375Hymenoscyphus fructigenus
FC-2676 "Hymenoscyphus herbarum"
FC-2793Hymenoscyphus pseudoalbidus
TN-S-F-12503Hymenoscyphus pseudoalbidus
FC-2799 Hymenoscyphus pseudoalbidus
FC-1445 Hymenoscyphus pseudoalbidus
TNS-F-52061 Hymenoscyphus pseudoalbidus
TNS-F-40074 Hymenoscyphus pseudoalbidus
TN-S-F-17817 Hymenoscyphus pseudoalbidus
FC-2137Lachnum virgineum
FC-2118Lachnum abnorme
95/-
100/100
100/100
58/51
100/100
94/99
100/100
100/100
89/-
100/-
98/-
77/80
100/100
83/-
99/100
90/-
95/97
98/91
99/99
100/100
20

origin has remained uncertain. Field inoculations showed that *Fraxinus ornus*, closely related to many Asian ash species, is less susceptible to infection than the European common ash, *F. excelsior* (Kräutler & Kirisits 2011), leading Queloz et al. (2011) to suggest a possible Asian origin for the ash dieback pathogen. In addition, *F. mandshurica*, a northeastern Asian ash ornamental introduced in Europe, was only slightly affected by ash dieback in southeastern Estonia (Drenkhan & Hanso 2010). It must be mentioned, however, that *F. mandshurica* is not closely related to *F. ornus* but is, instead, close to *F. excelsior* (Wallander 2008). Pathogenicity of *H. pseudoalbidus* to indigenous ash has not been reported in Japan.

To clarify the origin of the pathogen, population genetic studies based on polymorphic microsatellite markers, have shown that *H. pseudoalbidus* possesses low genetic variation among and within populations (Bengtsson et al. 2012, Gross et al. 2011). The genetic homogeneity of European *H. pseudoalbidus* can be explained by the founder effect (characteristic of invasive populations established by a small number of individuals). In the present study, Japanese *H. pseudoalbidus* showed a higher genetic variation than European populations, and Japanese populations were more basal in the phylogenetic analyses. Together with a very low susceptibility of local *Fraxinus*, this does not contradict an Asian origin for *H. pseudoalbidus*. In order to unravel the origin of *H. pseudoalbidus*, however, further genetic comparisons of populations from other Asian regions are needed.

Comparison of the ITS, CAL and EF1-α gene nucleotides has shown the European *H. pseudoalbidus* and *H. albidus* to be extremely stable and with clear differences in various positions. Although Japanese *H. pseudoalbidus* shared most characters with the European *H. pseudoalbidus*, some nucleotide positions varied. Especially in the EF1-α gene, strain FC-2799 appeared intermediate between *H. pseudoalbidus* and *H. albidus*. Records of *H. albidus* in Japan are unknown up to now, and the reason for the intermediate nucleotide patterns needs further study. Possibly the mixed nucleotide positions in Japanese materials suggests a common ancestor of *H. albidus* and *H. pseudoalbidus* from eastern Asia.

Hosoya et al. (1993) was the first to report the presence of an anamorphic structure in *H. pseudoalbidus*, based on Japanese material. At the time, the authors regarded the structure as spermatia due to its minute size and the fact that conidial germination was not observed. In the present study, the absence of conidial germination was again noted (on MEA). This suggests that the asexual state of *H. pseudoalbidus* does not contribute to its long-range dispersal and that pathogen dispersal is limited to ascospores, as previously suggested (e.g. Gross et al. 2011, Timmermann et al. 2011, Gross et al. 2012).

Lambertella was chiefly characterized by the presence of a substratal stroma and ascospores that may become brown at various stages of spore development (Dumont 1971, Korf & Zhuang 1985). Our current phylogenetic analysis (Fig. 5) places *Lambertella* species in both of the two major clades, suggesting that the genus is not monophyletic. The molecular phylogeny of Holst-Jensen et al. (1997) also suggested polyphyly in *Lambertella.* Korf (1982) transferred *Hymenoscyphus albidus* to *Lambertella* because it has the major morphological characters of *Lambertella* (Hosoya et al. 1993). Both substratal stroma and brown ascospores were observed in *H. pseudoalbidus* by Kowalski & Holdenrieder (2009), but the authors retained this fungus in *Hymenoscyphus.*

Our phylogenetic analyses situate *H. pseudoalbidus* in the *Hymenoscyphus* group, suggesting that *Hymenoscyphus* is the more appropriate genus to accommodate this fungus at present. This is in concordance with morphological characteristics, such as the *Hymenoscyphus*-type of ascus apical ring and the heteropolar scutuloid ascospores in *H. pseudoalbidus* (Baral & Bemmann, in prep.). The *Hymenoscyphus* clade also contains *H. ginkgonis,* another species with a substratal stroma and ascospores becoming brown when germinated (although these characters were not mentioned by Han & Shin, 2008). In conclusion, the two major characters (presence of substratal stroma and brown ascospores) currently used to circumscribe the genus *Lambertella* seem to have resulted from convergent evolution, and the generic delimitation of *Lambertella* needs to be reconsidered.

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New records of *Scleroderma* species (*Sclerodermataceae, Agaricomycetes*) from Pakistan

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Abstract — During a survey of gasteroid basidiomycetes from Pakistan, three *Scleroderma* species (*S. areolatum, S. chevalieri, S. dictyosporum*) have been identified that represent new records from Pakistan. A key to the known *Scleroderma* species from Pakistan is presented.

Key words — *Boletales*, earth balls, fungal taxonomy, Khyber Pakhtun Khwa, Nathia gali, Neelum valley

Introduction

Scleroderma Pers. is an extensively studied genus (Guzmán 1970, Sims et al. 1997, Kasuya et al. 2002, Baseia & Galvão 2002, Yoshimi 2000, 2002). This genus comprises 30 species worldwide, most of which occur in North America (Guzmán 1970, Kirk et al. 2008). *Scleroderma* species are thick-skinned earth balls that are separated primarily based on basidiospore characters (Sims et al. 1995). These gasteroid basidiomycetes form ectomycorrhizal associations with various trees and have a definite opening from which they eject their spores (Marshall 1904, Jeffries 1999).

Five *Scleroderma* species have been recorded previously from Pakistan (Ahmad et al. 1997). Continuing the survey of gasteroid basidiomycetes from Pakistan (Khalid & Iqbal 1996, 2004, Sultan et al. 2001, Razzaq & Shahzad 2004, 2007, Iqbal et al. 2006, Sultana et al. 2007, Moreno et al. 2009), we collected and identified three more *Scleroderma* species, which we describe here as an addition to the gasteroid mycota of Pakistan.

Materials & methods

Slides were prepared by mounting a smear of spores in a drop of 5% KOH, lactophenol and trypan blue. Spore ornamentation was observed under oil immersion (100×) objective. Spore size was determined by measuring the diameter of at least forty spores

including the ornamentation and then calculating the mean. Only mature spores were selected for the measurements. Peridium thicknesses, glebal color, surface ornamentation of basidiomata were also observed using 10× hand lens. Drawings were made with the help of camera lucida and measurements were taken using ocular micrometer.

Taxonomy

Scleroderma areolatum Ehrenb., Sylv. mycol. berol. 15: 27 (1818) FIG. 1

BASIDIOMATA epigeous, ≤30 mm broad, 25 mm high, subglobose, attached to the substratum by a thick mycelial base, forming a pseudostipe; pseudostipe

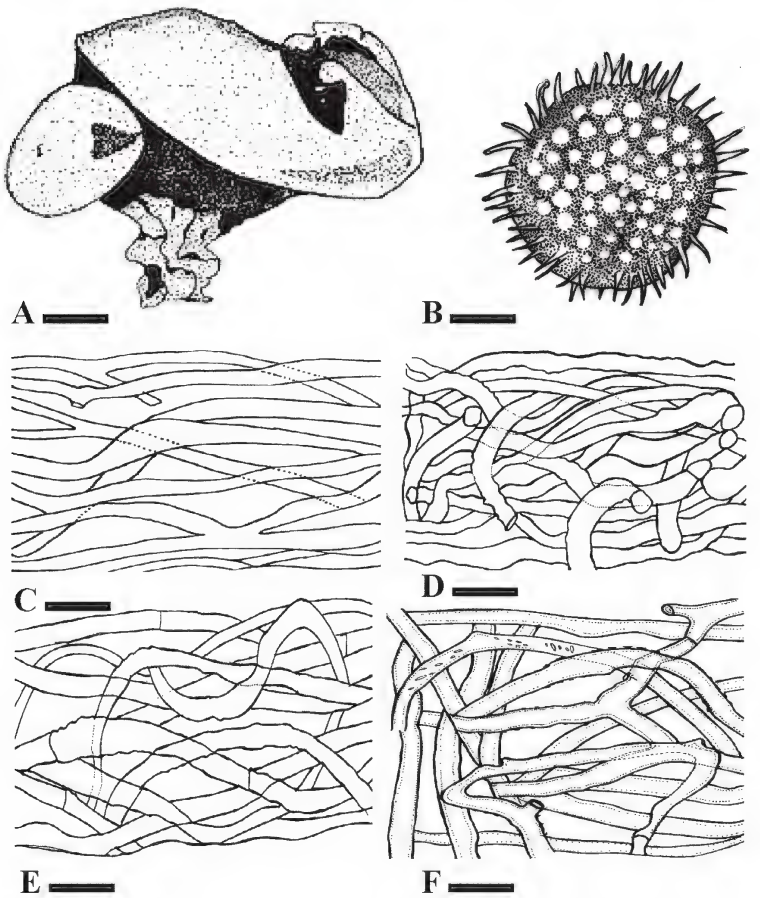

FIG. 1: *Scleroderma areolatum*. A. Basidiomata. B. Basidiospore. C. Exoperidial hyphae. D. Mesoperidial hyphae. E. Endoperidial hyphae. F. Tramal hyphae. Scale bars: A = 0.5 cm; B = 3.5 µm; C = 28 µm; D, F = 14 µm; E = 18 µm.

tough, often two basidiomata arise from the same tuft of mycelium. Rhizomorphs white, thick, growing individually or in small groups. Peridium thin, <1mm thick, pale yellow to brown; basal portion smooth, without scales, off white to pale yellow, upper part with brown spots like scales, dehiscence by tearing away the apical portion. Gleba powdery, dark purplish.

Basidiospores brown, globose, echinulate, never reticulate, 12–18 µm in diam., echines ≤2.5 µm long. Tramal Hyphae rarely septate, branched, hyaline, with few amorphous material encrusted, ≤5.5 µm wide, wall thickness ≤1 µm. Exoperidium composed of aseptate, branched hyphae, ≤7 µm wide, wall thickness ≤2.5 µm. Mesoperidium composed of aseptate, branched hyphae, wall encrusted, ≤7 µm wide, wall thickness 2.6 µm. Endoperidium composed of septate, branched hyphae, ≤6.6 µm wide, wall thickness ≤2.8 µm.

Specimen examined: **PAKISTAN: Khyber Pakhtun Khwa**, Khanspur, Ayubia, ca. 2575 m a.s.l., solitary on ground, in Himalayan moist temperate forest, 12 Aug. 2007, N. Yousaf NYG04 (LAH 120807).

Comments: The ectomycorrhizal *S. areolatum* is a poisonous species (Yamada & Katsuya 1995, Mason et al. 2000, Chen et al. 2006a,b) that is characterized by non-reticulate, densely echinulate basidiospores, a distinctive diagnostic feature, as other *Scleroderma* spp. examined have reticulate and sub-reticulate basidiospores. *Scleroderma areolatum* is widely distributed in Asia, Australia, Europe, North America, and South America (Lu et al. 1999, Giachini et al. 2000, Kasuya et al. 2002, Barroetaveña et al. 2005, Chen et al. 2006b).

Scleroderma chevalieri Guzmán, Ciencia Méx. 25: 202 (1967) Fig. 2

Basidioma epigeous, 45 mm broad, 40 mm high, subglobose, attached to the substratum via a tuft of mycelium, latter some time aggregated to form pseudostipe. Rhizomorphs white, thick. Peridium <1 mm thick, hard, rough, covered with patches of scales, mature specimen cracked at the upper portion, dehiscence by the irregular splitting of the peridium. Gleba pulverulent, pale yellow, brown to olivaceous.

Basidiospores brown, globose, echinulate, partially reticulate or sub reticulate, 11–17 µm in diam., echines ≤3 µm long. Tramal Hyphae aseptate, branched, hyaline, with clamp connections, ≤5.3 µm wide, wall thickness ≤1.3 µm. Exoperidium composed of septate, unbranched hyphae; ≤8.8 µm wide, wall thickness ≤2.0 µm. Mesoperidium composed of aseptate, unbranched hyphae, clamp connections rarely present; ≤9.5 µm wide, wall thickness ≤2.4 µm. Endoperidium composed of aseptate, unbranched hyphae, ≤9.5 µm wide, wall thickness 2.5 µm.

Specimen examined: **PAKISTAN: Khyber Pakhtun Khwa**, Nathia Gali, ca. 2501 m a.s.l., in groups, on ground, under *Abies pindrow* in Himalayan moist temperate forest, 25 Aug. 2006, N. Yousaf NYG03 (LAH 250806).

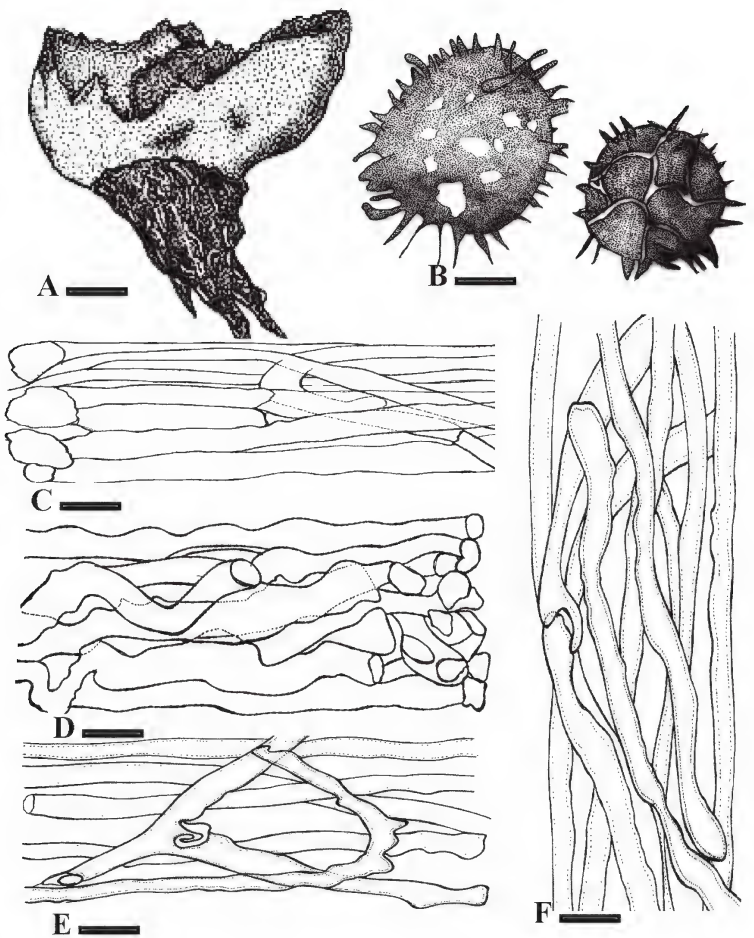

Fig. 2: *Scleroderma chevalieri.* A. Basidiome (weathered). B. Basidiospores. C. Exoperidial hyphae. D. Mesoperidial hyphae. E. Tramal hyphae. F. Endoperidial hyphae. Scale bars: A = 0.8 cm; B = 3.5 µm; C = 16 µm; D = 18 µm; E = 10 µm; F = 24 µm.

Comments: Like other members of *Scleroderma*, *S. chevalieri* has a hard, tough peridium and is characterized by a subglobose basidioma with short pseudostipe. It has large ornamented basidiospores with a sub-reticulum and peridium ≤1 mm thick. *Scleroderma sinnamariense* Mont. and *S. stellatum* Berk., also have a similar peridial thickness and partially reticulate spores. However, *S. sinnamariense* has a colored peridium with lemon yellow to black scales and smaller spores, and *S. stellatum* has a star-shaped opening and smaller spores (Sims et al. 1995).

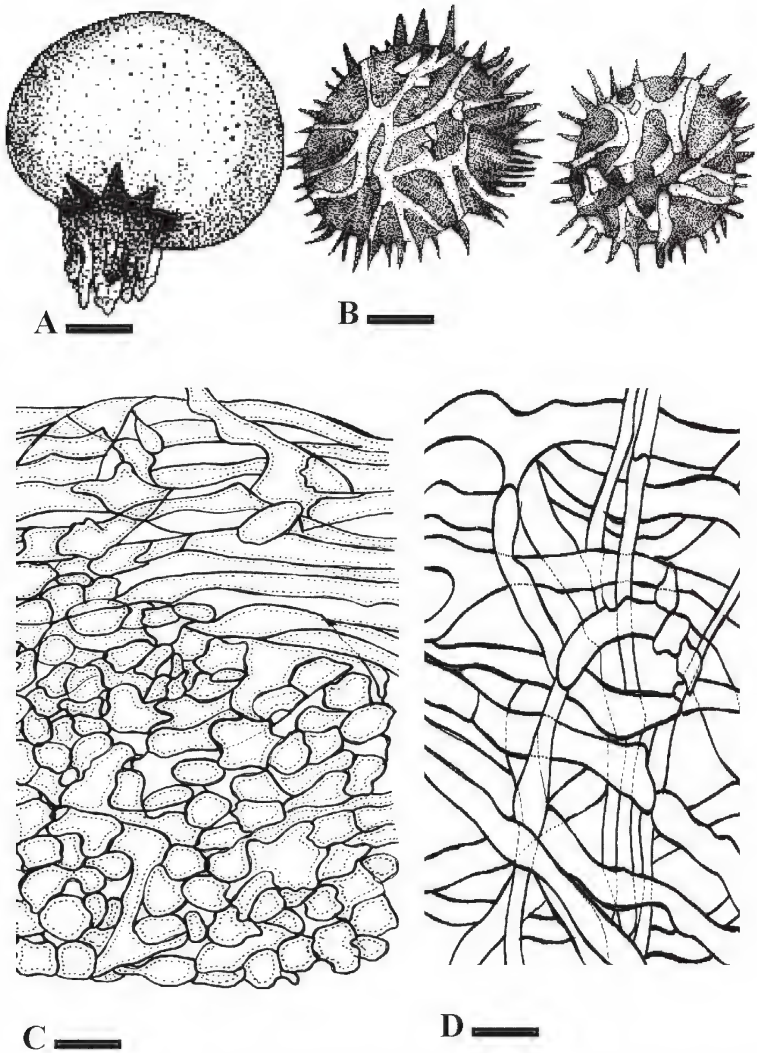

FIG. 3: *Scleroderma dictyosporum*. A. Basidiome. B. Basidiospores. C. Exoperidial and mesoperidial hyphae. D. Endoperidial hyphae. Scale bars: A = 0.6 cm; B = 3.5 µm; C = 28 µm; D = 10 µm.

Scleroderma dictyosporum Pat., Bull. Soc. Mycol. Fr. 12: 135 (1896) FIG. 3

BASIDIOMATA epigeous, ≤30 mm broad and 30 mm high, globose to subglobose, dull brown, with well-developed mycelial base often produced into a pseudostipe; several fruit bodies arising from a single stalk. PERIDIUM <1 mm thick, tough, relatively smooth, elastic, brittle in dried specimen cuticle

breaking into scales; scales black, thin, denser on top, spot-like; dehiscence by an irregular rupturing of an apical part. Gleba firm, compact, powdery with age, grayish to sepia, lighter towards the center.

Basidiospores brown, globose, echinulate-reticulate, 12–15 µm in diam., echines ≤4 µm long, young spores without reticulum, nurse cells prominent in the mount. Tramal hyphae hyaline, thin-walled, with clamp connections. Exoperidium composed of fascicles of erect hyphae, dark orange to brown, ≤6.5 µm, thick-walled, encrusted. Mesoperidium with globose elements, hyaline (yellowish in mass), ≤6 µm in diam., thin-walled, mixed with long hyaline hyphae. Endoperidium composed of septate, branched, hyaline hyphae, surrounding the gleba, hyphae ≤6 µm wide, thin-walled.

Specimen examined: **PAKISTAN: Khyber Pakhtun Khwa**, Naran (Kaghan valley), ca. 3350 m a.s.l., solitary on sandy soil, 23 Sep. 2009, N. Yousaf NYG01 (LAH 230909).

Comments: *Scleroderma dictyosporum* is an ectomycorrhizal species (Mukerji et al. 2000, Rai et al. 2009) and is distinguished by its globose basidioma with shorter pseudostipe, thin peridium (<1mm), echinulate-reticulate basidiospores (12.5–15.0 µm) with echines ≤4 µm. This species is close to *S. fuscum* (Corda) E. Fisch., but the latter has smooth peridium, absence of stipe and larger basidiospores (≤17 µm).

Key to *Scleroderma* species from Pakistan

1a. Basidiospores reticulate 2
1b. Basidiospores echinulate or verrucose, never reticulate 5
2a. Basidiospores partially to subreticulate 3
2b. Basidiospores completely reticulate 4
3a. Basidiospores 11–17 µm in diam., echines <3.0 µm long ..... *S. chevalieri*
3b. Basidiospores 5–8 µm in diam., echines <1µm long ..... *S. sinnamariense*
4a. Basidiospores 12–15 µm in diam., echines <4 µm long ..... *S. dictyosporum*
4b. Basidiospores 11–16 µm in diam., echines <1 µm long ..... *S. bovista*
5a. Basidiospores 8–13 µm 6
5b. Basidiospores larger, 10–18 µm 7
6a. Basidiospores 8–13 µm, echines >1 µm long ..... *S. verrucosum*
6b. Basidiospores 8.5–12 µm, echines <1 µm long ..... *S. cepa*
7a. Basidiospores 12–18 µm, echines >2.5 µm long ..... *S. areolatum*
7b. Basidiospores 10–14 µm, echines ≤1.5 µm long ..... *S. flavidum*

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Aphyllophoroid fungi from Sonora, México 2. New records from Sierra de Álamos–Río Cuchujaqui Biosphere Reserve

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Abstract — *Gyrodontium sacchari, Leiotrametes menziesii, Phellinus glaucescens*, and *P. shaferi* are described as new records from México. The specimens were collected on dead or living wood in tropical deciduous forest in the Sierra de Álamos–Río Cuchujaqui Biosphere Reserve located in Sonora State, México.

Key words — *Trametes*, taxonomy, chorology

Introduction

The Sierra de Álamos–Río Cuchujaqui Biosphere Reserve has an extension of 92,899 ha and is located in south of State of Sonora, México. It presents an extraordinary biological richness, with approximately 1100 species of plants distributed in xerophilous scrub, tropical deciduous forest and oak-pine forest (CONANP 2007). The aphyllophoroid fungi are heterogeneous groups of several taxa of macrofungi that are very abundant in this region, mainly on live and dead wood. In this study four species are described for the first time for México: *Gyrodontium sacchari* (*Coniophoraceae, Boletales*), *Phellinus glaucescens* and *P. shaferi* (*Hymenochaetaceae, Hymenochaetales*), and *Leiotrametes menziesii* (*Polyporaceae, Polyporales*).

Materials & methods

The examined specimens were collected from Sonora State in September 2006 and are deposited in ENCB Herbarium with duplicate in CESUES. Herbarium ENCB is

abbreviated according to Thiers (2012). Morphological examinations followed protocols outlined by Ryvarden (1991) and Cifuentes et al. (1986). Keys in parentheses after colors in basidioma descriptions follow the Methuen Handbook of Colour (Kornerup & Wanscher 1978). Measurements of anatomical characters were taken from rehydrated tissues in 5% aqueous KOH and amyloid reactions were taken with Melzer's reagent. Longitudes and latitudes were obtained with GPS etrex (garmin). Line drawings were made to scale using a camera lucida attached to a light microscope. The macro-photos were taken with a Nikon Coolpix 4300.

Taxonomy

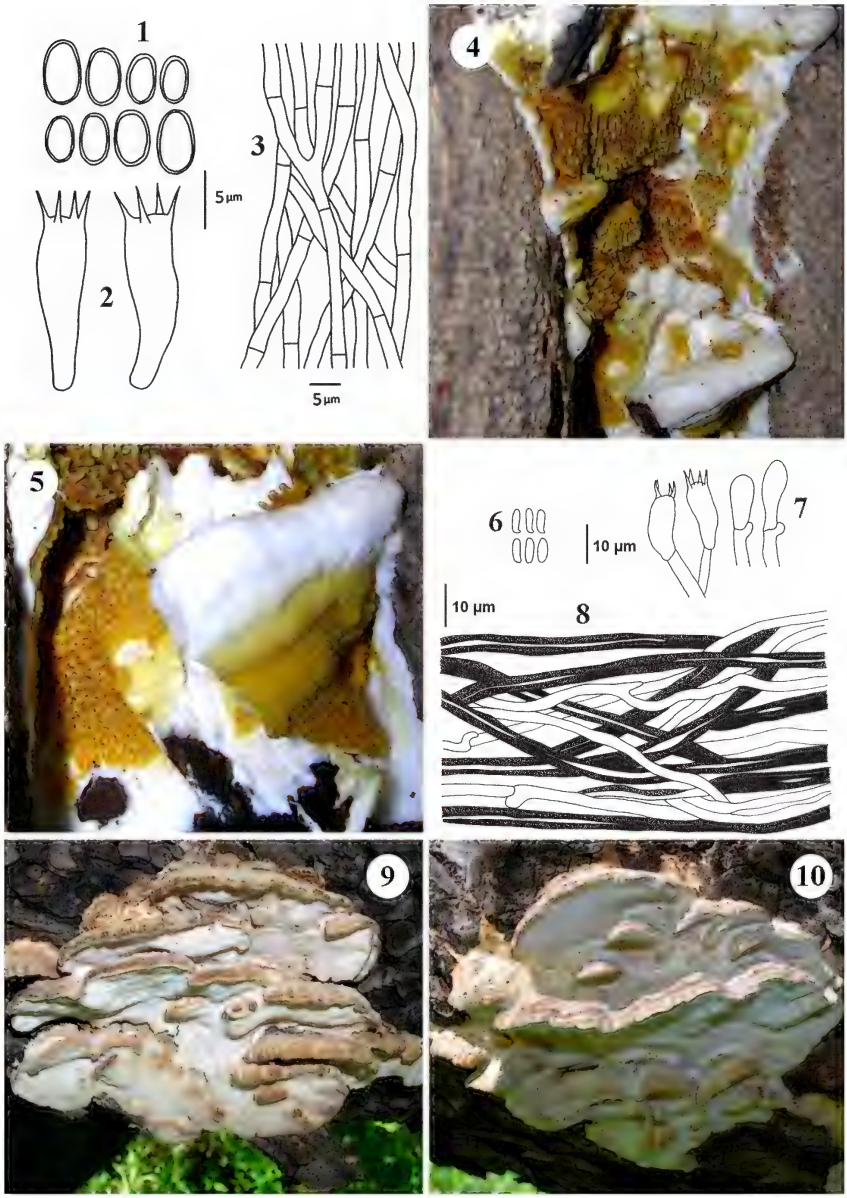
Gyrodontium sacchari (Spreng.) Hjortstam, Mycotaxon 54: 186, 1995. FIGS 1–5

BASIDIOMES annual, 40–50 mm broad and up to 100 mm long, resupinate to effuse-reflexed, subpileate to pileate, fleshy to spongy, easily separable from the substratum. PILEUS poorly to well development, conchate in some parts, soft to spongy, white. MARGIN obtuse and thick in pileus, acute at effused parts, sterile, membranaceus, spongy to cottony, white, width, up to 12 mm. HYMENOPHORE tuberculate appearance in young parts, aculeate to distinctly hydnoid in mature parts; aculei mainly flattened, some cylindrical, up to 5 mm long, yellow (2-3A8), sulphur yellow (1A5), olive yellow (2-3D8) to olive green (3D7), reddish-brown (8E8) when the spores covering the surface. CONTEXT or subiculum up to 5 mm thick, soft to spongy, cottony, white.

HYPHAL SYSTEM monomitic; GENERATIVE HYPHAE simple septate, hyaline to yellowish in KOH, inamyloid, simple to branched, mainly thin-walled, some thick-walled, 2.4–8 µm diam. HYMENOPHORAL TRAMA with parallel to subparallel hyphae, hyaline to yellowish in KOH, simple to slightly branched, thin-walled, 2.4–4.8 µm diam. CONTEXTUAL HYPHAE slightly interwoven, hyaline in KOH, simple to branched, thin to thick-walled, 4–8 µm diam. CLAMP CONNECTIONS absent. CYSTIDIA absent. BASIDIA 10.8–15 × 4–6.4 µm, clavate, hyaline in KOH, tetra-spored, sterigmata up to 3 µm long. BASIDIOSPORES 4–5.6 × 2.4–3.2 µm, ellipsoid to elongate, yellowish brown in KOH, inamyloid to weak dextrinoid, thick-walled, smooth.

ECOLOGY & DISTRIBUTION— *Gyrodontium sacchari* grows on live legume wood in tropical deciduous forests, causing a brown rot. Carlier et al. (2004) mentioned that this pantropical species has been recorded on all continents. It is known from Ethiopia, Tanzania, and Zimbabwe in Africa (Hjortstam 1987, Carlier et al. 2004), Thailand (Carlier et al. 2004) in Asia, from Guadeloupe, Brazil (Hjortstam 1995) and French Guyana (Carlier et al. 2004) in South America. In México it was found in the municipality of Álamos, Sonora State.

REPRESENTATIVE SPECIMEN EXAMINED — **MÉXICO. SONORA: municipality of Álamos**, Palo Injerto (27°02′50.9″N 108°43′57.9″W) elev. 425 m, 13.IX.2006, R. Valenzuela 13068 (ENCB, CESUES).



Figs 1–10: ***Gyrodontium sacchari***: 1. Basidiospores. 2. Basidia. 3. Hyphae of hymenophoral trama. 4–5. Basidiomes. ***Leiotrametes menziesii***: 6. Basidiospores. 7. Basidia. 8. Hyphae of hymenophoral trama. 9–10. Basidiomes.

Comments— *Gyrodontium sacchari* is characterized by a resupinate to an effuse-reflexed basidiome, yellow to olive green hydnoid hymenophore, monomitic hyphal system with simple septate generative hyphae, and small thick-walled smooth yellowish brown basidiospores. The monotypic *Gyrodontium* can be confused with *Serpula* species, but the meruloid hymenophore and clamped generative hyphae easily separate both genera (Hjortstam 1987). Phylogenetic analysis indicates that *Gyrodontium* and *Coniophora* share clampless generative hyphae and are related, but *Coniophora* has a smooth hymenophore and forms exclusively resupinate basidiomes (Carlier et al. 2004).

Leiotrametes menziesii (Berk.) Welti & Courtec., Fungal Diversity 55: 60, 2012

Figs 6–10

Basidiomes annual, 60–120 x 30–70 x 10–30 mm, pileate-sessile, imbricate, connate, corky in consistency. pileus dimidiate to broadly attached, conchate, semicircular, velutinous to glabrous, azonate or with narrow zones to the margin, whitish, pale yellow (4A2, 4A3) to ochraceous yellow (5C7) with brownish grey (5D7) in young specimens, ochraceous yellow (5C7), orange grey (5B2), grayish orange (5B3), brown (6E4) with spots grayish brown (6E3), brownish gray (6E2) to gray (6E1) in mature specimens. margin sterile, thin, acute, lobed in some specimens, recurved, whitish to brown (6E8) or dark brown (7F4); pale gray (5D1) to black in dry specimens. hymenophore poroid, white to cream color (4A3), with tones straw yellow (4A4) in some parts, gray (5D1) or pale brown (6D3) in others; pores rounded to irregular in shape, 5–7 per mm, with the dissepiments entire to slightly lacerated; tubes up to 10 mm deep, concolorous with the pores. context up to 20 mm thick, homogeneous, dense, zonate, white to pale yellow (4A2, 4A3).

Hyphal system trimitic, generative hyphae with clamp connections, hyaline in KOH, inamyloid, simple to branched, thin-walled, 2.4–3.2 µm in diam.; skeletal hyphae hyaline in KOH, inamyloid, unbranched, thick-walled to solid, 3.2–6.4 µm in diam., some swollen up to 10 µm broad; binding hyphae hyaline in KOH, inamyloid, thin-walled to thick-walled, very branched, 2.4–4 µm diam. hymenophoral trama with interwoven hyphae, generative hyphae hyaline, thin-walled, simple to slightly branched, 2.4–3.2 µm wide; skeletal hyphae yellowish hyaline, unbranched, thick-walled, 3.2–5.6 µm broad; binding hyphae hyaline in KOH, inamyloid, thin-walled to thick-walled, very branched, 2.4–4 µm diam. contextual hyphae very interwoven, generative hyphae hyaline, simple to branched, thin-walled, 2.4–3.2 µm wide; skeletal hyphae hyaline, unbranched, thick-walled to solid, 3.2–6.4 µm in broad; binding hyphae hyaline in KOH, inamyloid, thin-walled to thick-walled, very branched, 2.4–4 µm diam. sterile cells absent. basidia 12–16 × 4.8–6.4 µm, clavate, tetra-spored, hyaline in KOH, with sterigmata up to 4 µm long.

BASIDIOSPORES 4.4–6.4 × 1.5–2.4 µm, cylindrical, hyaline in KOH, inamyloid, thin-walled, smooth.

ECOLOGY & DISTRIBUTION— *Leiotrametes menziesii* grows on dead legume wood in tropical deciduous forests and causes a white rot. Ryvarden & Johansen (1980) regard this species, widely distributed in Africa, Asia, Australia, and Pacific Islands, as paleotropical. It has been cited (as *Trametes menziesii*) from Malaysia (Corner 1989), Papua New Guinea (Quanten 1997), and (in South America) Costa Rica (Carranza & Ruiz-Boyer 2005), Guyana (Aime et al. 2003), and Martinique (Welti et al. 2012). The most northernmost report of *L. menziesii* is from two sites in Sonora state, México.

REPRESENTATIVE SPECIMEN EXAMINED — **MÉXICO. SONORA: municipality of Álamos**, El Aguaje (26°56′45.9″N 108°45′48.9″W) elev. 450 m, 14.IX.2006, R. Valenzuela 13106 (ENCB, CESUES); El Platanar, (26°59′26.7″N 108°40′40.4″W) elev. 635 m, 14.IX.2006, R. Valenzuela 13137 (ENCB, CESUES).

COMMENTS— *Leiotrametes menziesii* is very variable in basidiome size, shape, and color, but it can be distinguished by the numerous gray tones (mainly towards the base) in mature specimens and spore size. Quanten (1997) mentioned the size differences for basidiospores cited by Cunningham (1965), Ryvarden & Johansen (1980), and Corner (1989), and the Mexican specimens are very close to or within the range given by these authors. The morphological features of Mexican specimens agree with those given by Ryvarden & Johansen (1980) and Quanten (1997).

Phellinus glaucescens (Petch) Ryvarden, Norw. Jour. Bot. 19: 234, 1972. FIGS 11–15

BASIDIOMES perennial, 80–100 × 45–60 × 5–8 mm, resupinate, becoming widely effused, adnate, corky. MARGIN sterile, up to 1 mm wide, dark brown (6F5) to black with age, matted, fimbriate. HYMENOPHORE poroid, cracked with age, PORES circular to angular, 6–8(–9) per mm, grayish brown (7D3-4) (8D3), light brown (6D8), golden brown (5D7), yellowish brown (5E7), cocoa brown (6E6), umber (6F6) to dark brown (6F7), iridescent, light brown (6D5) when moved, edges thin and entire; TUBES up to 7 mm deep, indistinctly stratified, tough to woody, yellowish brown (5E7), cocoa brown (6E6) to reddish brown (8E8). CONTEXT or subiculum up to 1 mm thick, yellowish brown (5E8) to reddish brown (8E8), fibrous, tough, azonate.

HYPHAL SYSTEM dimitic, GENERATIVE HYPHAE simple septate, hyaline to pale yellow in KOH, simple to slightly branched, thin-walled, 1.6–3.2 µm in diam; SKELETAL HYPHAE yellowish brown to reddish brown, unbranched or with rare branched, thick-walled, 3.2–5.6 µm in diam. HYMENOPHORAL TRAMA with parallel to subparallel hyphae, generative hyphae hyaline to pale yellow in KOH, thin-walled to thick-walled, simple to slightly branched, 2.4–4 µm wide; skeletal hyphae yellowish brown to reddish brown in KOH, unbranched,

thick-walled, 3.2–5 µm in diam. CONTEXTUAL TRAMA with slightly interwoven hyphae, generative hyphae hyaline in KOH, simple to scarcely branched, thick-walled, 1.6–2.4 µm wide; skeletal hyphae pale yellow in KOH, unbranched, thick-walled, 2.4–3.2 µm in diam. HYMENIAL SETAE 14-20 × 4.8-6.4 µm, ventricose to ventricose-rostrate, reddish brown to dark brown, thick-walled. BASIDIA 10–14 × 5.6–6.4 µm, clavate, tetra-spored, hyaline in KOH. BASIDIOSPORES 4–4.8 × 3.2–4.0 µm, broadly ellipsoid to subglobose, pale yellow to yellowish brown in KOH, inamyloid, slightly thick-walled, smooth.

ECOLOGY & DISTRIBUTION— Solitary, growing on dead angiosperm wood in tropical deciduous forests. This species is also known from Africa and Asia (Ryvarden & Johansen 1980, Larsen & Lombard 1988, Larsen & Cobb-Poulle, 1990). The first record from the Americas is in México, where *P. glaucescens* was found in the State of Sonora.

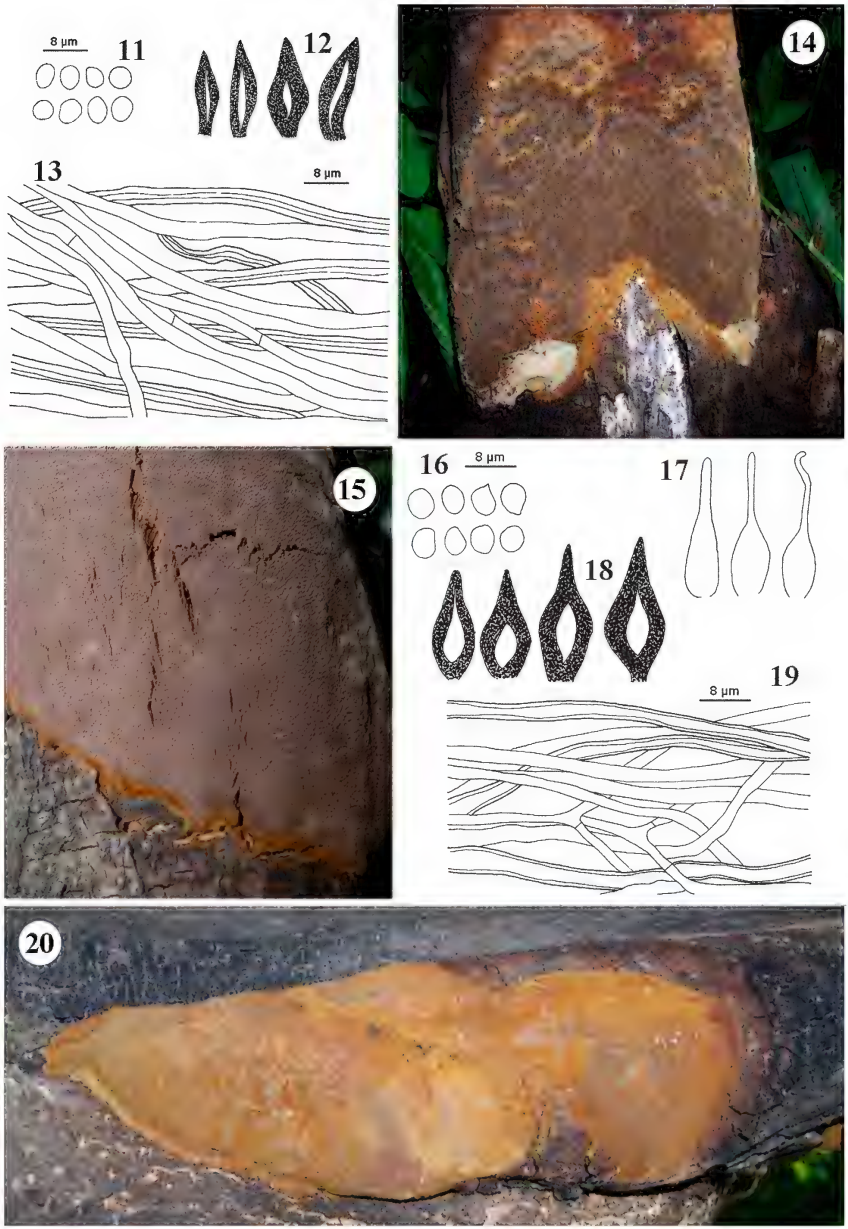
REPRESENTATIVE SPECIMEN EXAMINED — **MÉXICO. SONORA: municipality of Álamos,** El Sabinito (27°00′5.5″N 108°48′14.2″W) elev. 377 m, 16.IX.2006, R. Valenzuela 13173, 13175 (ENCB, CESUES).

COMMENTS— Within *Phellinus, P. glaucescens* belongs to the resupinate species complex, from which it is separated by the combination of basidiome color, small pores, the presence of hymenial setae, and spore size, shape, and color. A similar species that has been cited from the area, *Inonotus tropicalis* (M.J. Larsen & Lombard) T. Wagner & M. Fisch. (Raymundo et al. 2009), has a resupinate basidiome, small pores, and similar basidiospores but is distinguished by its annual to biennial fruiting pattern, fasciculate or clustered hymenial setae, and a monomitic to pseudodimitic hyphal system.

Phellinus shaferi (Murrill) Ryvarden, Norw. Jour. Bot. 19: 235, 1972. FIGS 16–20

BASIDIOMES perennial, 90–140 × 40–70 × 4–7 mm, resupinate, widely effused, adnate, tough to woody. MARGIN sterile, up to 1 mm wide, brownish yellow (5C7), golden brown (5D7) to light brown (6D8), dark brown (6F5) to black with age, matted, fimbriate. HYMENOPHORE poroid, cracked with age, PORES circular to angular, 5–6 per mm, yellowish brown (5E7) to reddish brown (8E8), dark brown (6F7) with age, edges thin and entire; TUBES up to 3 mm deep, tough to woody, yellowish brown (5E7) to reddish brown (8E8). CONTEXT or subiculum up to 1 mm thick, yellowish brown (5E8) to reddish brown (8E8), fibrous, tough, azonate.

HYPHAL SYSTEM dimitic, GENERATIVE HYPHAE simple septate, hyaline to pale yellow in KOH, simple to slightly branched, thin-walled, 2.4–3.2 µm in diam; SKELETAL HYPHAE golden brown to reddish brown, unbranched, thick-walled, 2.4–5.0 µm in diam. HYMENOPHORAL TRAMA with slightly interwoven hyphae, generative hyphae hyaline to pale yellow in KOH, thin-walled, simple to slightly branched, 2.4–3.2 µm wide; skeletal hyphae golden brown to reddish



Figs 11–20: ***Phellinus glaucescens***: 11. Basidiospores. 12. Hymenial setae. 13. Hyphae of hymenophoral trama. 14–15. Basidiomes. ***Phellinus shaferi***: 16. Basidiospores. 17. Cystidioles. 18. Hymenial setae. 19. Hyphae of hymenophoral trama. 20. Resupinate basidiome.

brown in KOH, unbranched, thick-walled, 3.2–5 µm in diam. CONTEXTUAL TRAMA with slightly interwoven hyphae, generative hyphae hyaline to pale yellow in KOH, simple to scarcely branched, thick-walled, 1.6–2.4 µm wide; skeletal hyphae yellowish brown in KOH, unbranched, thick-walled, 2.4–4.0 µm in diam. HYMENIAL SETAE 16–22.4 × 5.6–9.6 µm, ventricose to ventricose-rostrate, reddish brown to dark brown, thick-walled. CYSTIDIOLES 24–26 × 7.2–8.8 µm, subulate to ventricose-rostrate, hyaline in KOH. BASIDIA not observed. BASIDIOSPORES 4–4.8 × 3.2–4.0 µm, subglobose, pale yellow to rusty brown in KOH, inamyloid, thin-walled, smooth.

ECOLOGY & DISTRIBUTION — Solitary, growing on dead angiosperm wood in tropical deciduous forests. Previously known from Montserrat in the West Indies, Panama, and Venezuela (Ryvarden 2004), in México *P. shaferi* was found in Sonora State.

REPRESENTATIVE SPECIMEN EXAMINED — **MÉXICO. SONORA: municipality of Álamos**, El Aguaje (26°56′45″N 108°45′48″W) elev. 462 m, 14.IX.2006, R. Valenzuela 13099 (ENCB, CESUES).

COMMENTS— *Phellinus shaferi* is characterized by its resupinate basidiomes, pore size and shape, and basidiospore size and color. It is separated from *P. glaucescens* by small pores (6–8 per mm). We follow Ryvarden (2004) in placing this taxon in *Phellinus*, until molecular studies confirm whether it would be better retained in *Fuscoporella*.

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Passalora acrocomiae sp. nov. and *Exosporium acrocomiae* from the palm *Acrocomia aculeata* in Puerto Rico

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Abstract — Collections determined by J.A. Stevenson as *Cercospora acrocomiae* [≡ *Exosporium acrocomiae*] from leaf spots of *Acrocomia media* [= *A. aculeata*] in Puerto Rico were re-examined. Two different fungi were identified: a previously unnoticed authentic cercosporoid taxon that we describe herein as *Passalora acrocomiae* and *Exosporium acrocomiae*, which we illustrate here for the first time and for which we designate a lectotype to substitute for the missing holotype.

Key words —*Arecaceae*, phytopathogens, reappraisal, taxonomic novelties

Introduction

Acrocomia aculeata (Jacq.) Lodd. ex Mart. (*Arecaceae*) is a thorny medium sized neotropical palm (Scariot & Lleras 1995). We present a reappraisal of the available specimens of a fungus collected on *A. media* [= *A. aculeata*] in Puerto Rico at the beginning of the 20th century. This dematiaceous fungus has been placed in two disparate genera, *Cercospora* and *Exosporium*. Firstly a description was given by Stevenson (1917) of the fungus as *Cercospora acrocomiae*. Chupp (1954) later described the same fungus, annotating it as "plainly an *Exosporium*." Accepting Chupp's opinion, Stevenson (1975) recombined the taxon as *Exosporium acrocomiae*. The original Stevenson and Chupp descriptions are very similar and fit best into the present concept of *Exosporium*. A re-examination of the original material was undertaken to confirm its placement in that genus.

Materials & methods

All relevant specimens were obtained as loans from BPI and CUP herbaria. Samples were examined under a dissecting microscope and freehand sections of fungal structures

or fungal structures scraped from colonized plant surfaces were mounted in lactic acid or lacto-fuchsin. Observations of fungal structures and measurements, as well as preparation of line drawings and photographs, were performed with an Olympus BX 51 light microscope fitted with a drawing tube and an Olympus E330 digital camera. Plates were organized with CorelDraw X5. Wherever possible, 30 measurements of structures from each fungus were made.

Taxonomy

Exosporium acrocomiae (J.A. Stev.) Chupp ex J.A. Stev., Contrib. Reed Herb. 23: 516, 1975. PLATE 1

MYCOBANK MB477989

≡ *Cercospora acrocomiae* J.A. Stev., Ann. Rep. Insular Exp. Stat. Porto Rico 1916–17: 89, 1917.

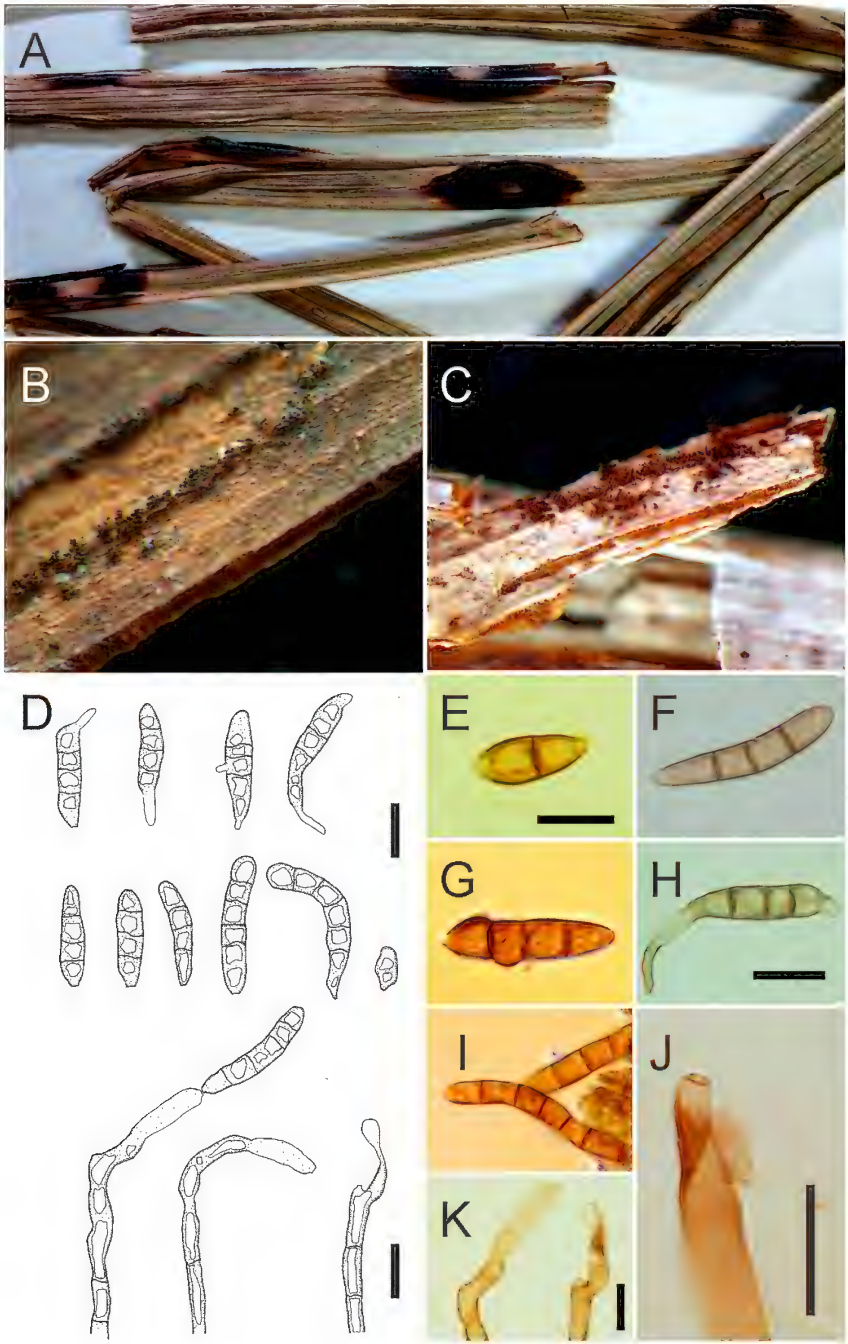
ORIGINAL DESCRIPTION (Stevenson 1917: 89–90): "Primary spots amphigenous, few, 1–8 per pinnæ: but areas between finally dying so as to make large continuous areas: regular, oval, with definite margins neither raised nor sunken, .8–1.5, rarely 2 cm. long by 3–6 mm. broad, rarely wider, at first red brown, then tricoloured, a central oval gray area (.5–3 mm. × 2–6 mm.), enclosed by a dark-brown band, 2–3 mm. wide, with an outer more or less irregular red-brown area, often no completely encircling the central portions. Conidal [sic] fascicles hypophyllous, numerous, confined to the central gray area, often in two distinct lines; conidiophores many to each fascicle, brown to olive brown, 1–2 septate, erect to reclining, concolorous with the conidia, 32–70 × 5–8 mu. Conidia 3–9 septate, long clavate, sometimes slightly curved, apical cell rounded, brown, often constricted at the septæ, 55–120 × 8–12 mu."

SUPPLEMENTARY DESCRIPTION (Chupp 1954: 428): "Pinnae of older leaves are affected, so that part or the whole leaf may finally be killed, single spots 8–20 × 3–6 mm., or coalescing into large irregular areas, often with a central oval gray area enclosed by a dark brown band, with an outer irregular reddish brown area; fruiting chiefly hypophyllous; stromata dark to black, 40–100μ in diameter; fascicles very dense; conidiophores dark fuligeneous brown, septate, not geniculate, straight, curved, or tortuous, sometimes wider near the tip or irregular in width, not branched, spore scars absent or indistinct, 4–8 × 30–70μ; conidia dark fuligeneous brown, straight to slightly curved, clavato-cylindric to slightly attenuated toward the tip, obtuse ends, septa distinct, 3–9 in number, 5–10 (812) × 30–120 μ."

TYPE: PUERTO RICO, Rio Piedras, on leaves of *Acrocomia media* [= *A. aculeata*], July 1917, J.A. Stevenson 6604 (**Holotype**, not located, presumed lost); 14 Feb. 1912, J.R. Johnston [Stevenson 4206, 4206A] (Paratype = **Lectotype designated here**, BPI 432400; **isolectotypes**, BPI 432402, CUP 039018).

Lesions on leaves subcircular to elliptic, with pale brown edges and paler centre, 1.2–2.0 × 0.3–0.60 cm. Internal mycelium indistinct. External mycelium absent. Stroma sub-epidermal, subglobose, erumpent, composed of dark brown textura angularis, smooth. Conidiophores hypogenous, in dense fascicles (of up to 20 conidiophores per fascicle), sub-cylindrical, straight to slightly

PLATE 1. *Exosporium acrocomiae.* A: Leaf spot symptoms on leaves; B–C: Hypogenous caespituli; D: Conidia and conidiophores; E–I: Conidia; J–K: Detail of conidiogenous loci. Bars: 20 µm.


A
B
C
D
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K

curved, occasionally geniculate at the apex, 40–70 × 4–5 µm, 3–5 (mainly 3)-septate, unbranched, thick-walled, pale brown to olivaceous-brown, smooth. Conidiogenous cells terminal, integrated, sub-cylindrical, 10–32 × 5–7 µm, pale brown, smooth. Conidiogenous loci almost inconspicuous, one per cell, 1.0–2.0 µm diam, thickened, not darkened. Conidia solitary, cylindrical, 30–100 × 5–10 µm, truncate at the base, apex rounded, 3–9-distoseptate, 2–3 µm diam at base, guttulate, pale brown to olivaceous-brown, smooth.

Additional Material Examined: **PUERTO RICO**, Rio Piedras, on *Acrocomia media*, 29 Jan. 1917, J.A. Stevenson 6175 (BPI 432399); 15 Jul. 1914, J.A. Stevenson No. 2090 (BPI 432397, 432398, 432401).

Passalora acrocomiae Guatimosim & R.W. Barreto, **sp. nov.** Plate 2

MycoBank MB801218

Differs from *Passalora eitenii* by its denser cespituli, stromata of textura intricata, smaller conidiophores, and absence of percurrently proliferating conidiogenous cells.

Type: Puerto Rico, Rio Piedras, on leaves of *Acrocomia media* [= *A. aculeata*], 15 Jul. 1914, J.A. Stevenson No. 2090 (**Holotype**, BPI 432397; **isotypes**, BPI 432398, 432401).

Etymology: from the host genus.

Lesions on leaves subcircular to elliptic, with pale brown edges and paler centre, 0.90–2.30 × 0.3–0.60 cm. Internal mycelium branched, 2.5 µm diam., septate, subhyaline. External mycelium absent. Stroma sub-epidermal, erumpent, subglobose, 100–157 × 87–113 µm, composed of dense textura intricata, pale brown, smooth. Conidiophores hypogenous, in dense fascicles (up to 20 conidiophores per fascicle), confined to the central gray area, often in two parallel distinct lines, sub-cylindrical, straight to slightly curved, 90–105 × 5–7 µm, 1–3-septate, unbranched, eguttulate, pale brown, smooth but with a distinctly roughened apex. Conidiogenous cells terminal, integrated, sub-cylindrical, 37–55 × 6–8 µm, pale brown, roughened. Conidiogenous loci conspicuous, one per cell, discoid, 1.5–4 µm diam. Conidia solitary, subcylindrical to slightly clavate, obconically truncate at the base, apex rounded, 35–68 × 8–13 µm, 1–3-septate, diam 2–4 µm at base, hila somewhat thickened and darkened-refractive, guttulate, pale brown to olivaceous-brown, mostly smooth but with a distinctly roughened apex.

Discussion

Based on examination of the holotype (Stevenson 6604) and other original collections, Chupp (1954) indicated that Stevenson's *Cercospora acrocomiae* was "plainly an *Exosporium*," but did not publish a valid new combination. Stevenson (1975) accepted Chupp's opinion, and published the valid combination *Exosporium acrocomiae*. The genus *Exosporium* Link (type species, *E. tiliae* Link) is characterized by having typically darkened, thickened and prominent conidiogenous loci (scars) and conidial hila. After careful examination of the

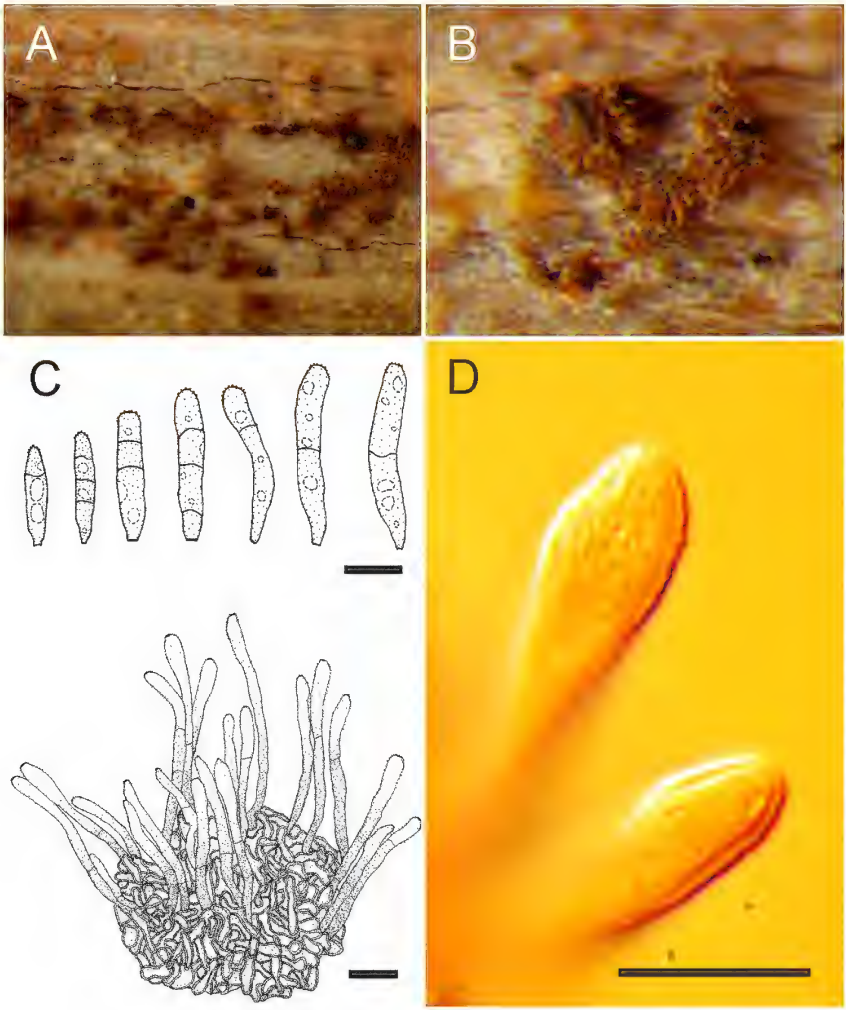

Plate 2. *Passalora acrocomiae.* A–B: Hypogenous caespituli; C–D: Conidia and conidiophores. Bars: 20 µm; E: Detail of the upper part of a conidiophore (note distinctly roughened apex). Bar: 5 µm.

original collections of *C. acrocomiae* (all from the same locality, including paratypes collected in 1912 and 1914 and an additional collection in 1917), we concluded that, in fact, two different fungi are present associated with the leaf spots on *A. aculeata* in Puerto Rico.

One is ubiquitous and is recognized as the fungus described by both authors, although neither Stevenson nor Chupp published illustrations of their fungus.

Although this fungus does not have the prominent, dark and thickened scars of many other *Exosporium* species, it still is adequately placed in *Exosporium*.

The second fungus associated with the *A. aculeata* leaf spots represents a novel species unnoticed by Stevenson and Chupp. This fungus is present only in three herbarium specimens from one Stevenson paratype collection, where it is much less common than *E. acrocomiae*. This new taxon bears the typical features of cercosporoid fungi now placed, under the current concept for this group, in *Passalora* (Crous & Braun 2003). Hence, the source of the confusion that emerged during the present study was the combined occurrence of two fungi on the same kind of lesions on one paratype collection of *C. acrocomiae*.

An additional complication is that, to the best of our knowledge, the holotype of *E. acrocomiae* is now lost; a search in all potential deposit sites for the holotype (BPI, CUP, ILL, ILS, UPRRP) failed to uncover this critical type specimen. Therefore, we chose one of the two paratypes (Stevenson 4206 = BPI 432400) as lectotype for *E. acrocomiae*.

It is interesting that a similar leaf spot was collected in Brazil during an ongoing study of the mycobiota of *A. aculeata* involving a third hyphomycete — also belonging to *Passalora* but clearly distinct from *P. acrocomiae* (pers. obs.). Probably this palm species reacts to attack by different pathogenic fungi by forming similar leaf spots.

Two *Exosporium* species have already been described on members of the *Arecaceae*: *Exosporium pulchellum* Sacc. on *Areca catechu* L. and *Orania palindan* (Blanco) Merr., both in the Philippines (Trotter 1931: 994–995; Teodoro 1937); *E. stilbaceum* (Moreau) M.B. Ellis on *Elaeis guineensis* Jacq. in Africa and Asia; and *Elaeis* sp. in Africa (Ellis 1971; Turner 1971; Pirozynski 1972; Liu 1977). These two species have much longer conidiophores (≤220 µm long) and thicker scars. Additionally *E. stilbaceum* forms conidia in chains and conidiophores arranged in synnemata (Ellis 1971).

There is only one *Passalora* described on the *Arecaceae*: *P. eitenii* R.B. Medeiros & Dianese on the Brazilian native palm *Syagrus comosa* (Mart.) Mart. *Passalora eitenii* differs morphologically *P. acrocomiae* by its sparser caespituli, stromata of textura angularis, larger conidiophores, and percurrently proliferating conidiogenous cells (Medeiros & Dianese 1994).

The search for a clarification of the status of *Exosporium* on *A. aculeata* resulted in confirming its identity in this genus and the unexpected discovery of an overlooked species of *Passalora*.

Acknowledgments

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A new *Stigmella* species associated with *Lycium* leaf spots in northwestern China

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Abstract — A new anamorphic species *Stigmella lycii* on *Lycium chinense* is described and illustrated from Chinese material and compared with all known species of this genus. This is the first report of *Stigmella* species in China.

Key words — coelomycete, taxonomy

Introduction

While investigating medical plant diseases in Gansu province, China, during 2004 and 2011, a species of *Stigmella* Lév. was discovered that caused large, circular, necrotic leaf spots on *Lycium chinense* Mill. in Jingtai county. Severe infections caused by this pathogenic fungus usually resulted in premature leaf and fruit fall. Based on careful morphological examination, we determined that this pathogen represented a new species of *Stigmella*.

Materials & methods

The fungal specimens were cut by hand with a razor blade to make thin sections of conidiomata for microscopical observation. The sections were mounted in water and lactophenol cotton blue on slides. Morphological characteristics of pycnidia, conidiogenous cells and conidia were carefully examined using light microscopy. For scanning electron microscope (SEM) examinations, shortly after collection 5 mm squares of fresh leaf tissue containing conidiomata were fixed overnight at 4°C in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2), washed with glutaraldehyde buffer, dehydrated in a 50-70-80-90-100% ethanol series for 15 min each, dehydrated in acetone, critical point dried, sputter-coated with gold, and examined in a JSM-6380 operating at 30 kV. The examined specimens were deposited in the Herbarium of Gansu Agriculture University (HGAU).

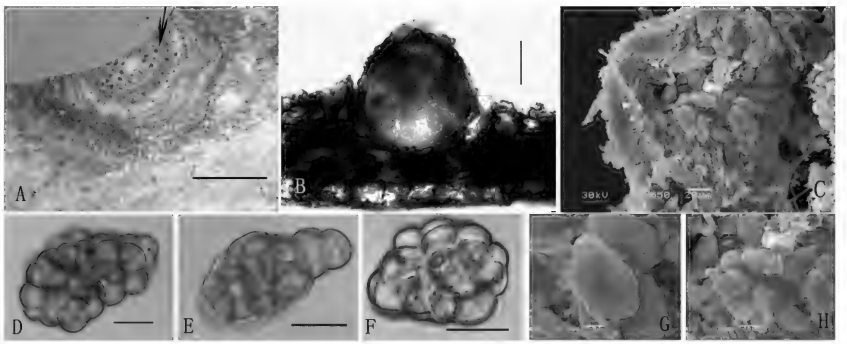

Plate 1. *Stigmella lycii*. A. Leaf lesion. Arrow indicating a single stroma. B–C. Section through a pycnidium with a layer of conidiogenous cells bearing conidia (B, by light microscopy; C, by SEM). D–F. Muriform conidia, by light microscopy. G–H. Muriform conidia, by SEM. Bars: A = 400 μm; B = 50 μm; C–F = 8 μm.

Taxonomy

Stigmella lycii X.R. Chen & Yan Wang, **sp. nov.** Plate 1.

MycoBank MB 800236

Differt ab omnibus speciebus Stigmellae pycnidiis *distincte majoribus et conidiis quoque majoribus.*

Type: China, Gansu province, Jingtai county, Damiao village, on leaves of *Lycium chinense* Mill. (*Solanaceae*), 22 October 2011, X.R. Chen & Y. Wang (**Holotype**, HGAU, Herbarium of Gansu Agriculture University).

Etymology: Epithet derived from the host genus.

Early lesions on leaves visible as small, round, pale brown leaf spots, gradually becoming larger necrotic blotches, somewhat zonate, with distinct margins, 2–8 mm diam. Lower leaves falling prematurely due to severe infections. Mycelium entirely immersed in the leaf tissue, septate, branched, hyaline to pale brown. Conidiomata pycnidial, immersed, erumpent at maturity, subglobose, blackish, 161–172 μm diam, with thin walls up to 10 μm diam, composed of one to three layers of small, pale brown to dark brown cells and an inner textura prismatica with thin-walled paler cells. Ostiole indefinite. Conidiogenous cells holoblastic, determinate, discrete, doliiform to ampulliform, hyaline, smooth, formed from the inner cells of the pycnidial wall. Conidia hyaline when young, at maturity pale olive-brown and muriform, oval, ellipsoid, pyriform, irregular and mulberry-shape, 19.9–52.8 × 12.8–32.9 μm, composed of 12–35 cells. Conidial cells small, irregular, subglobose, pale yellowish, 2.6–11.5 × 2.6–11.0 μm.

Discussion

The coelomycetous genus *Stigmella* has an intricate history. Its type species, *S. dryina* Lév. (Demidow 1842), was placed in the 'Phyllostictei' by Fuckel

(1870), along with representatives of *Coniothyrium* Corda, *Phoma* Sacc., *Darluca* Castagne, and other genera. Saccardo (1878) assigned '*Stigmella dryina* (Corda) Lév.' (an incorrect author citation) to hyphomycetes, a practice followed by subsequent authors. Bubak (1914) was the first to recognize the pycnidial structure of *S. dryina*. Hughes (1952), who conducted the first modern revision of *Stigmella* based on its type species, concluded from original descriptions that most species attributed to this genus were hyphomycetes and not congeneric with *S. dryina*. Sutton (1980) published a later description of the genus.

The two other species accepted in *Stigmella* are *S. effigurata* (Schwein.) S. Hughes (= *S. dryina*; = *S. dryophylla* (Corda) Lindau; see Hughes 1958) and the Indian *S. tirumalensis* Bagyan. et al. (Bagyanarayana et al. 1992). Based on conidioma structure, conidiogenesis, and conidial morphology, the recently collected Chinese fungus can clearly be allocated to *Stigmella* where it represents a third species, *S. lycii*, that is easily distinguished from the other species by much larger pycnidia and conidia with a distinctive cellular composition (Table 1).

Table 1. Comparison of *Stigmella effigurate, S. tirumalensis,* and *S. lycii*

	S. effigurata	*S. tirumalensis*	*S. lycii*
Pycnidia	≤100 µm	40–100 µm, rarely ≤200 µm	172–162 µm
Conidia	12–20 × 7–10 µm; 6 transverse + variable longitudinal septa	10–14 (–21) × 6–9 µm; ≤4 transverse & 0–3 longitudinal septa	20–53 × 13–33 µm, comprising 12–35 small cells (2.6–11.5 × 2.6–11 µm)
Host/ distribution	Leaves of *Quercus* spp., *Atriplex suberecta*, *Chenopodium* sp., *Eugenia* sp., *Senecio mesogrammoides*, *Stephania abyssinica* / Africa, Asia, Europe	Leaves of *Cassine glauca*/ India	Leaves of *Lycium chinense* / China

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Tuber sinoaestivum sp. nov., an edible truffle from southwestern China

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Abstract — A new *Tuber* species, *T. sinoaestivum*, is described from southwestern China based on morphological and molecular analyses. The new species, which was found fruiting in association with *Pinus armandii*, has been previously misidentified as *T. aestivum* (=*T. uncinatum*). Although morphologically quite similar, *T. sinoaestivum* differs from the European species by more globose ascospores ornamented with a shallower reticulum. ITS sequence analyses show only 92% similarity between the two species, further supporting the taxonomic separation of *T. sinoaestivum* from *T. aestivum*.

Key words —black truffles, *Pezizales*, phylogeny, taxonomy

Introduction

Chinese black truffles have attracted much scientific and commercial interest since their export to Europe in the beginning of 1990s. Four black truffle taxa have previously been reported from China: *Tuber indicum* complex, *T. formosanum*, *T. pseudohimalayense*, and *T. aestivum* (Chen et al. 2005; Chen & Liu 2011; Hu 1992; Manjon et al. 2009; Song et al. 2005; Wang et al. 1998, 2006; Zhang et al. 2005). The high morphological similarity between Chinese and European black truffles has prompted intensive investigations on species recognition and phylogenies (Bonito et al. 2010; Douet et al. 2004; Jeandroz et al. 2008; Paolocci et al. 1997; Roux et al. 1999). Previous studies indicate that a number of major *Tuber* clades are distributed across both China and Europe, yet species within the two regions appear to be distinct from each other.

T. aestivum Vittad. (= *T. uncinatum* Chatin), a renowned culinary species in Europe, is widely distributed across Nordic and Mediterranean regions (Jeandroz et al. 2008). This species was reported as a new Chinese record

based on ascomata sold at mushroom markets in Huidong, Sichuan (Chen et al. 2005; Song et al. 2005). Both identifications relied on morphological comparison with European collections, and spore size and ornamentation differences were regarded as mere intraspecific variations. In their worldwide phylogeny of *Tuber*, Jeandroz et al. (2008) did not include Asian *T. aestivum* material, leaving open the true identity of the Chinese collections. In recent years, more *T. aestivum*-like ascomata have been found in southwestern China. Morphological and ITS-rDNA and β-tubulin sequence comparisons of these Chinese truffles with European collections indicate that the Chinese material differs from *T. aestivum* and should be described as a new species.

Materials & methods

Morphological examination

Macroscopic characters are based on fresh and dried specimens. Descriptions and spore parameters follow Yang & Zhang (2003). Slides were made with a razor blade from dried ascomata and mounted in 5% KOH. Micro-morphological features were examined under a Nikon E400 microscope (10 × 100). At least 100 spores were measured from each ascoma and included asci containing different numbers of spores. For scanning electron microscopy (SEM), ascospores were scraped from dried ascoma gleba and mounted in distilled water on a cover glass. After air drying the cover glasses were directly attached to a SEM stub with double-sided tape and then coated with gold-palladium. The treated materials were examined and photographed with an AMRAY 1000B SEM. All specimens cited are deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Science (KUN-HKAS).

Molecular methods

Six truffle collections from China and seventeen from Europe were selected for phylogenetic analysis (Table 1). Total DNA was extracted from gleba dried with silica-gel using a modified CTAB procedure of Doyle & Doyle (1987). The ITS5 (forward) and ITS4 (reverse) primers were used to amplify the internal transcribed spacer regions (ITS-rDNA) of the nuclear ribosomal DNA (White et al. 1990) and Bt2a (forward) and Btspect (reverse) primers were used for the β-tubulin gene (Glass et al. 1995; Paolocci et al. 2004). The amplifications used 2 µl DNA template solutions in final volume of 25 µl. The final solution contained 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of each primer and 3 U Taq Polymerase (Takara Biotechnology, Dalian Co. Ltd., China). The cycling parameters were an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C (for ITS and 49 °C for β-tubulin) for 90 s and extension at 72 °C for 2 min, then a final extension at 72 °C for 10 min. PCR products were visualized through gel electrophoresis on a 1% agarose gel and purified with Watson's purification kit. Sequencing was performed with ABI Prism BigDye terminator cycle sequencing kit v3.1 on an ABI PRISM 3730 automatic sequencer. DNA sequences were edited and aligned with BioEdit Version 5.0.9 (Hall 1999) and Clustal X with manual adjustment where necessary. Maximum parsimony (MP) analysis was conducted with PAUP* 4.0b4a (Swofford, 2002) using heuristic

Table 1 *Tuber* sequences used in phylogeny.
(Bold font = sequences newly generated in this study.)

Voucher	GenBank label	Identification	GenBank		Locality
			# ITS	# β-tubulin	
——	*T. malenconii*	*T. malenconii*	FM205597	——	Spain
——	*T. malenconii*	*T. malenconii*	FM205596	——	Spain
E2	*T. aestivum*	*T. aestivum*	AF516781	AF516801	Italy
E61	*T. aestivum*	*T. aestivum*	AF516783	AF516812	Italy
E60	*T. aestivum*	*T. aestivum*	AF516789	AF516804	Italy
E5	*T. uncinatum*	*T. aestivum*	AF516791	AF516809	Italy
E24	*T. uncinatum*	*T. aestivum*	AF516792	AF516807	Italy
E17	*T. uncinatum*	*T. aestivum*	AY226042	AY206632	Italy
HKAS44347	*T. aestivum*	*T. sinoaestivum*	GU979038	GU979152	China
HKAS44322	*T. aestivum*	*T. sinoaestivum*	GQ217542	GU979150	China
HKAS70291	***T. aestivum***	***T. aestivum***	**JQ348410**	**JQ348402**	**Sweden**
HKAS70292	***T. aestivum***	***T. aestivum***	**JQ348411**	**JQ348401**	**Sweden**
HKAS70296	***T. aestivum***	***T. aestivum***	**JQ348412**	**JQ348404**	**France**
HKAS70297	***T. aestivum***	***T. aestivum***	**JQ348413**	**JQ348403**	**France**
HKAS59111	***T. sinoaestivum***	***T. sinoaestivum***	**JN896352**	**JQ348409**	**China**
HKAS59100	***T. sinoaestivum***	***T. sinoaestivum***	**JN896356**	**JQ348408**	**China**
HKAS59101	***T. sinoaestivum***	***T. sinoaestivum***	**JN896357**	**JQ348406**	**China**
HKAS59102	***T. sinoaestivum***	***T. sinoaestivum***	**JN896358**	**JQ348407**	**China**
HKAS70294	***T. mesentericum***	***T. mesentericum***	**JQ348414**	**JQ348405**	**France**
M13	*T. mesentericum*	*T. mesentericum*	AF516793	AY170363	——
M1	*T. mesentericum*	*T. mesentericum*	AF516794	AY170364	——
G02	*T. magnatum*	*T. magnatum*	AJ586251	AJ586430	Croatia
B6	*T. magnatum*	*T. magnatum*	AJ586271	AJ586428	Italy

search with multi-trees setting on and tree bisection reconnection (TBR) branch swapping with 1000 search replicates, each replicate with random sequence addition. No ambiguous characters were excluded from the analyses. Gaps were treated as missing data. Character states were treated as equally weighted and unordered. *Tuber magnatum* was selected as outgroup based on previous analyses, and *T. sinoaestivum, T. aestivum*, *T. mesentericum*, and *T. malenconii* comprised the ingroup taxa.

Results

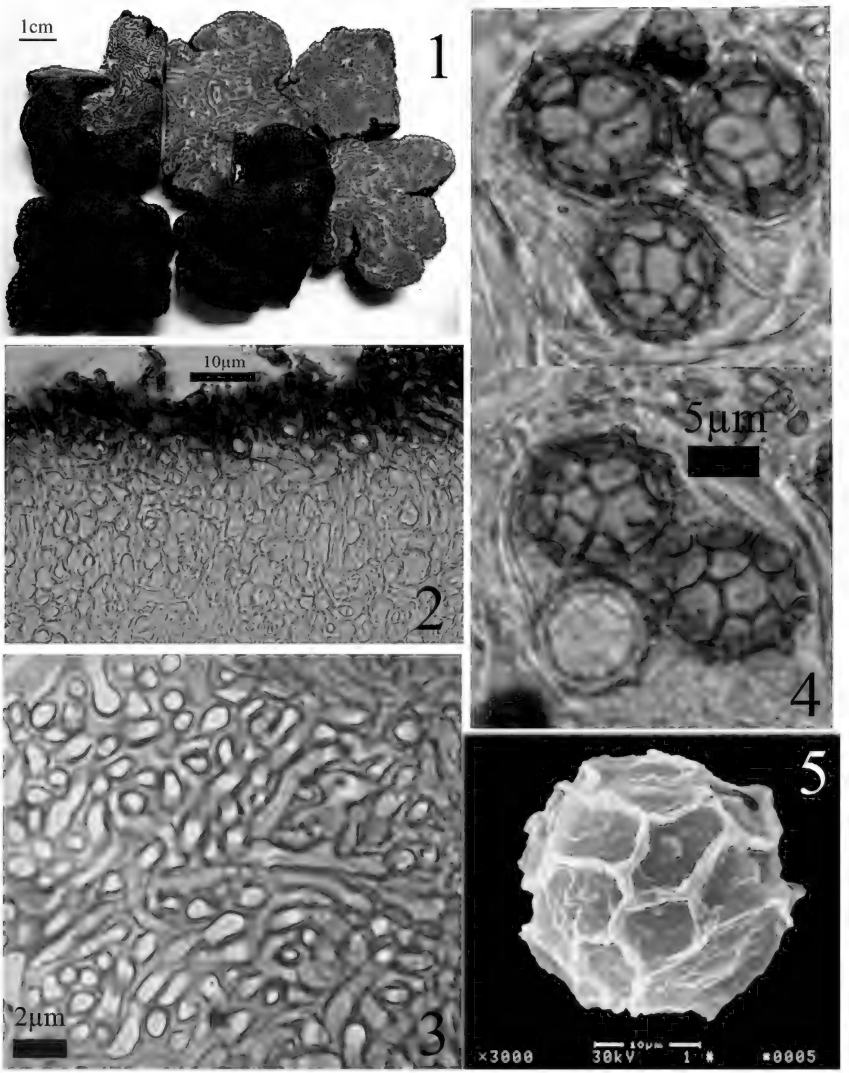
Taxonomy

Tuber sinoaestivum J.P. Zhang & P.G. Liu, **sp. nov.** Figs 1–5

MycoBank MB 563565

Differs from *Tuber aestivum* by its more globose spores with thicker walls and shallower ornamentation mesh.

Type: China, Sichuan Province, Huidong County, Gaji mountain, 26°29′N 102°31′E, alt. 2280 m, in *Pinus armandii* Franch. forest, 25 Nov. 2009, JP Zhang 153 (**holotype** KUN-HKAS 59105).



Figs 1–5. *Tuber sinoaestivum*. 1: Fresh ascomata; 2: Pseudoparenchymatous tissue of outer layer of peridium; 3: Intricately interwoven hyphae of inner layer of peridium; 4: Ascospores; 5: SEM of ascospore, showing the reticulate ornamentation.

Etymology: *sinoaestivum* (Lat.) referring to its Chinese origin and its relationship to European *T. aestivum*.

Ascomata subglobose, 1.5–3(–6) cm in diam., blackish to black, conspicuously covered with low, polygonal warts; warts 3–6-sided, 2–4 mm transverse, about 2 mm high, apex pointed. Peridium 100–620 μm thick, consisting of two

layers; the outer layer 50–300 µm thick, pseudoparenchymatous, composed of subglobose or subangular cells; cells dark to yellowish brown, 7–15 × 6–12 µm, thick-walled; the inner layer 50–350 µm thick, pale yellow or hyaline, interwoven hyphae 2–5 µm diam., forming an intricate texture. Gleba whitish at first when young, then becoming yellowish or brown gradually, marbled with numerous, narrow, white branching veins. Asci (51–)55–88(–124) × (33–)40–72(–75) µm (excluding stalk), subglobose, ellipsoid or variable in shape, sessile or with a short stalk, the stalk (6–)10–25(–50) × 5–15(–16) µm, 1–6(–7)-spored. Ascospores globose, subglobose, (17–)20–41(–47) × (15–)17–30(–35) µm [Q =1.04–1.50(–1.57), Q =1.15 ± 0.09], spore walls 2–4 µm thick, yellowish-brown at maturity, ornamented with a more or less irregular reticulum 2–5 µm deep; meshes variable, usually (7–)8–21(–22) × (4–)5–19(–20) µm, commonly 1–3 meshes in the transverse diam. and 2–4 meshes in the longitudinal direction. Odor unique with pleasant fragrance, taste a bit sweet for its mild aroma.

Habit and habitat: hypogenous, symbiotic with *P. armandii*, maturing from October to December or later.

Distribution: known only from southwestern China.

Additional specimens examined: ***Tuber sinoaestivum*** – **CHINA: Sichuan Province**, Huidong County, Daqiao mountain, 26°29′N 102°31′E, alt. 2250 m, 26 Nov. 2009, JP Zhang 158 (KUN-HKAS 59108); JP Zhang 156 (KUN-HKAS 59110); 19 Oct 2003, J Chen 129 (KUN-HKAS 44322). Gaji market, 23 Oct 2003, J Chen 141 (KUN-HKAS 44347). Gaji mountain, 26°29′N 102°31′E, alt. 2280 m, 25 Nov. 2009, JP Zhang 143 (KUN-HKAS 59095); JP Zhang 152 (KUN-HKAS 59104). Haiba mountain, 26°29′N 102°31′E, alt. 2300 m, 23 Nov. 2009, JP Zhang 140 (KUN-HKAS 59100); JP Zhang 141 (KUN-HKAS 59099); JP Zhang 144 (KUN-HKAS 59096); JP Zhang 149 (KUN-HKAS 59098). Yezu mountain, 26°29′N 102°31′E, alt. 2300 m, 27 Oct. 2009, JP Zhang 65 (KUN-HKAS 59101); JP Zhang 66 (KUN-HKAS 59102).

Tuber aestivum – **ENGLAND:** Hedben Bridge forest, 6 Aug. 2010, PG Liu (KUN-HKAS 70301, 70302). **FRANCE:** 2006, Y Wang (KUN-HKAS 70293, 70294, 70295); Lacoste, 46090 Le Montat, P Sourzat (KUN-HKAS 70296); La Devèze, 46090 Cours, P Sourzat (KUN-HKAS 70297). **ITALY:** Valle Fiorano (Modena), 10 Dec. 1984, A Zambonelli (KUN-HKAS 70298, 70299, 70300). **SWEDEN: Island of Gotland,** middle part of island, 8 Nov. 2003, C Wedén (KUN-HKAS 70290, 70291, 70292).

Phylogeny

Twenty-three samples were used for phylogenetic analysis. The homogeneity test for the conjoint analysis of ITS-rDNA and β-tubulin did not detect conflict between the ITS-rDNA and β-tubulin analyses, which allowed for a concatenated alignment and analysis. The alignment includes 1107 characters, of which 951 are constant, 149 are parsimony informative, and 7 are parsimony uninformative. Parsimony analysis generated one MP tree, with tree length = 360, CI = 0.928, RI = 0.968, RC = 0.898, and HI = 0.072.

In the concatenated analysis, the twenty-three samples grouped into three well-supported clades (Fig. 6): Clade I included all the Chinese materials

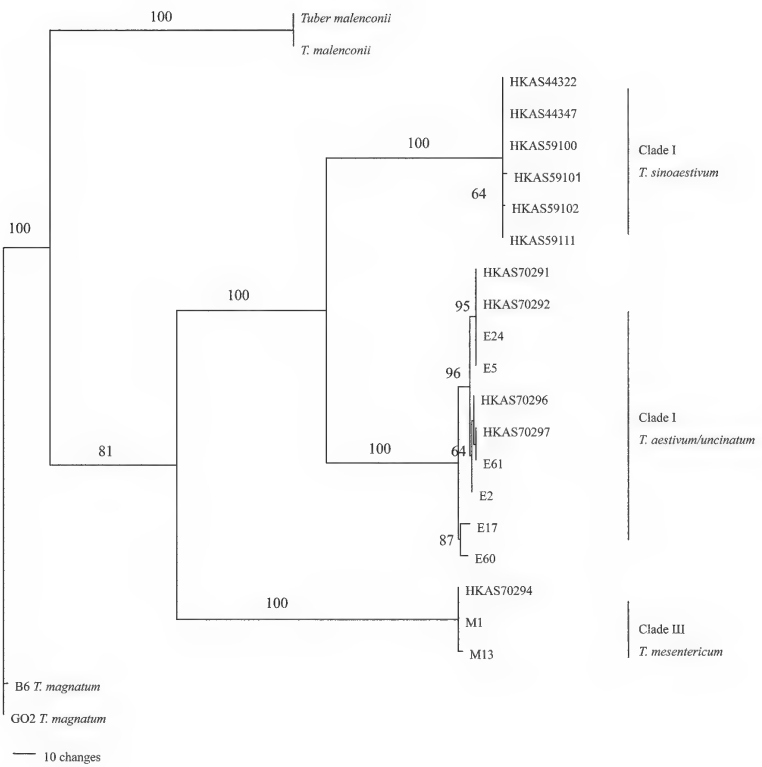


FIG. 6. Phylogenetic relationships of *Tuber sinoaestivum* and *T. aestivum* inferred from maximum parsimony (MP) analysis of ITS and β-tubulin sequences. MP Bootstrap values greater than 70% are indicated at nodes.

analyzed (*T. sinoaestivum*), Clade II included *T. aestivum/uncinatum* from Europe, and Clade III was represented by three *T. mesentericum* samples. Clade I formed a sister clade with Clade II. The same tree topology was obtained from the single gene ITS-rDNA and β-tubulin analyses but with different bootstrap values: the ITS-rDNA analysis shows 99% and 100% bootstrap support for the *T. aestivum/uncinatum* and *T. sinoaestivum* clades respectively, whereas the β-tubulin analysis indicates only 61% and 69% support. All analyses suggest a close relationship between *T. sinoaestivum* and *T. aestivum/uncinatum, T. mesentericum,* and *T. malenconii* but support a clear separation between the Chinese and the three European species.

Discussion

In the molecular phylogenetic tree, European *T. aestivum* collections (and others annotated as *T. uncinatum*) grouped together into clade II with 99% bootstrap support. The taxonomic relationship between *T. aestivum* and *T. uncinatum* has been subject to debate, with some regarding them as two species (Chevalier et al. 1994; Mello et al. 2002; Riousset et al. 2001) and others treating *T. uncinatum* as a morphological variation of *T. aestivum* (Sin et al. 1995; Trappe 1979), or perhaps two extremes of a morphological species-complex (Gandeboeuf et al. 1994; Pacioni et al. 1993; Urbaneli et al. 1998). Based on current research and our studies, we accept *T. aestivum* and *T. uncinatum* as a single widespread species with highly variable morphological and ecological characters. In addition, the phylogenetic trees suggest that "genetic variation" is not great. A previous multi-gene (ITS-rDNA, β-tubulin, EF1-α) analysis of sixty *T. aestivum* or *T. uncinatum* samples from Italy and other European countries has also supported *T. uncinatum* and *T. aestivum* as a single species (Paolocci 2004). A subsequent study by Wedén (2004) has drawn the same conclusion.

Tuber aestivum was initially reported from China as a new record because of its morphological resemblance to the European *T. aestivum* (Chen et al. 2005; Song et al. 2005). The Chinese collections that we studied are morphologically similar to the European *T. aestivum* in that both have black ascomata with conspicuous polygonal warts and reticulate spores. Such morphological similarity is mirrored in our ITS-rDNA and β-tubulin sequence analysis rooted by *T. magnatum* (Bonito et al. 2010; Jeandroz et al. 2008). However, after observing several European *T. aestivum* collections, we found that despite their almost identical macrocharacters, the two species are easily distinguished by spore shape. *Tuber sinoaestivum* spores are more globose (Fig. 7) as shown by the parameters in *T. sinoaestivum* [Q = (1.00–)1.04–1.40(–1.43), **Q** = 1.15 ± 0.11] in contrast to those in *T. aestivum* [Q = (1.03–)1.10–1.50(–1.65), **Q** =1.29 ± 0.13]. Furthermore, *T. sinoaestivum* spores have much thicker (2–4 μm) walls and shallower (<5 μm) meshes compared with the thinner walls (normally <2 μm thick) and higher (4–8 μm) meshes in *T. aestivum*. Chen et al. (2005) and Song et al. (2005) also noticed these differences but failed to use them for specific discrimination. European mycologists also noted the more globose spores in the Chinese *T. sinoaestivum,* but regarded it as a separate taxon (Zambonelli et al. 2012). Although *T. aestivum* is regarded as a widespread species with high morphological variation, the differences in *T. sinoaestivum* exceed the variation accepted for *T. aestivum.*

Until now, *T. sinoaestivum* has been found only from very limited localities in southwestern China. In the local Sichuan markets, it is often found mixed with collections representing the *T. indicum* complex. Zambonelli et al. (2012)

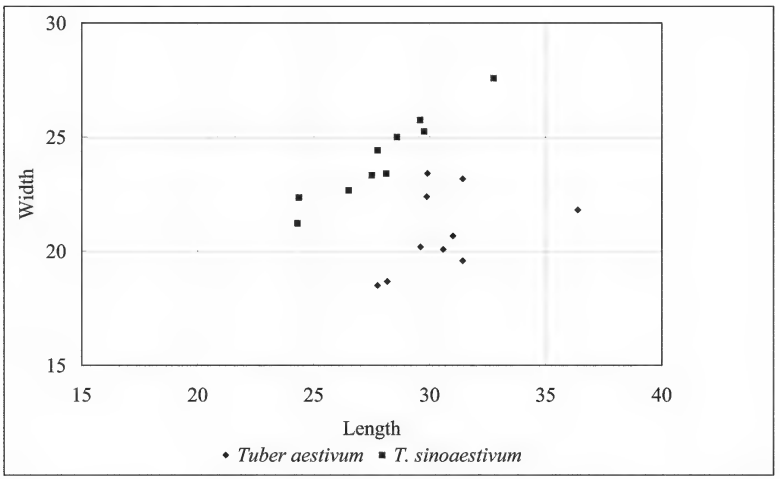


FIG. 7. Ascospore dimensions of *Tuber aestivum* and *T. sinoaestivum*; each point represents the mean values for 30 ascospores from a single ascocarp.

reported *T. sinoaestivum* (as "*T. aestivum* s.l.") as being sold in an Italian market. The very small number cited suggests that it could be mixed with fruiting bodies of *T. indicum* complex, a truffle intensively commercialized internationally. Potential introduction into *T. sinoaestivum* ecosystems cannot be excluded, as has occurred with *T. indicum* (Bonito et al. 2010; Murat et al. 2008), and precautions should be taken to avoid unwanted species invasions. Nevertheless, the culinary traits of *T. sinoaestivum* can still be commercially valuable. Accordingly, we suggest that when this truffle is traded, it should be classified and explicitly labeled *T. sinoaestivum* to emphasize its geographical origin and thus avoid confusion with *T. aestivum* and *T. indicum* complex. This will be significant for both the sustainable development and conservation of truffle biodiversity.

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Two new *Ellisembia* species from Hainan and Yunnan, China

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Abstract — *Ellisembia heritierae* sp. nov. on *Heritiera littoralis* and *E. pistaciae* sp. nov. on *Pistacia chinensis* are described and illustrated from specimens collected in tropical forests in Hainan and Yunnan Provinces, China. They are compared with closely related taxa.

Key words —anamorphic fungi, systematics, taxonomy

Introduction

Subramanian (1992) established *Ellisembia* Subram. typified by *E. coronata* (Fuckel) Subram. to accommodate those taxa previously described in *Sporidesmium* that have distoseptate conidia borne terminally on determinate or percurrently extending conidiogenous cells. The genus *Imicles* Shoemaker & Hambl. was later merged into *Ellisembia* by Wu & Zhuang (2005). More than 40 *Ellisembia* species have been described, mostly from rotten wood and dead branches of various plants (Subramanian 1992, McKenzie 1995, 2010, Goh & Hyde 1999, Mena-Portales et al. 2000, Zhou et al. 2001, Wu & Zhuang 2005, Heuchert & Braun 2006, Ma et al. 2008, 2011b).

The lignicolous mycota is very rich in tropical and subtropical forests of Hainan and Yunnan Provinces (Dai & Cui 2006, Dai & Li 2010, Ma et al. 2010, 2011a, Cui et al. 2011, Zhang et al. 2011). During ongoing surveys of tropical forest microfungi in the two regions, two new *Ellisembia* species were found. The type specimens are deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP) with isotypes in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

Ellisembia heritierae S.C. Ren & X.G. Zhang, **sp. nov.** Fig. 1

MycoBank MB 800477

Differs from *Ellisembia ellipsoidea* in its wider conidia with fewer distosepta and non-extending conidiogenous cells.

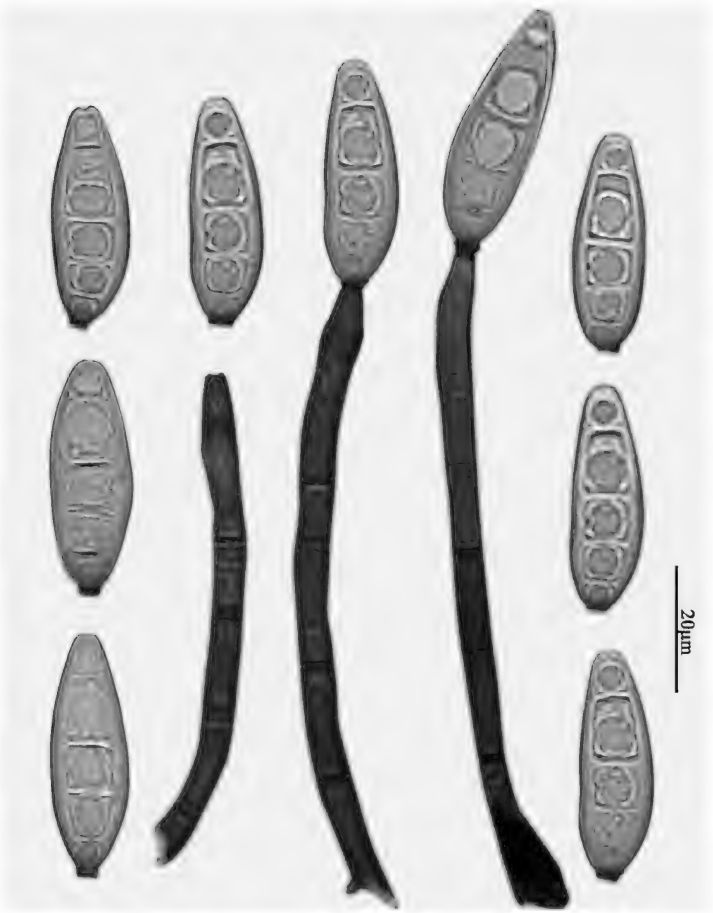

Fig. 1. *Ellisembia heritierae*. Conidiophores, conidiogenous cells and conidia.

Type: China. Hainan Province: tropical forest of Xishuangbanna, on decaying branches of *Heritiera littoralis* Aiton (*Sterculiaceae*), 30 October 2011, Sh.C. Ren (**Holotype** HSAUP H0062; **isotype** HMAS 243417).

Etymology: in reference to the host genus, *Heritiera*.

Colonies on natural substrate effuse, dark brown to black, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown, smooth-walled hyphae, 1.5–3 μm wide. Conidiophores distinct, single or in groups, erect, unbranched, straight or flexuous, cylindrical, brown, smooth, 4–7-septate, 80–110 μm long, 5–6.5 μm wide. Conidiogenous cells monoblastic, integrated, terminal, cylindrical, brown, smooth, determinate. Conidial secession schizolytic. Conidia holoblastic, solitary, acrogenous, ellipsoidal, rounded at the apex, truncate at the base, smooth-

walled, brown to pale brown, 4-distoseptate, 35–40 µm long, 12–14.5 µm wide in the broadest part, 2.5–3.5 µm wide at the truncate base.

Comments – *Ellisembia heritierae* resembles *E. ellipsoidea* W.P. Wu in producing ellipsoidal conidia, but can be differentiated from *E. ellipsoidea* (conidia 40–45 × 10–11 µm, 7–8-distoseptate) by its wider conidia with fewer distosepta. In addition, conidiogenous cells of *E. ellipsoidea* have up to 3 lageniform to ampulliform percurrent extensions, while those of *E. heritierae* have no extensions.

Fig. 2. *Ellisembia pistaciae.* Conidiophores, conidiogenous cells and conidia.

Ellisembia pistaciae S.C. Ren & X.G. Zhang, **sp. nov.** Fig. 2

MycoBank MB 800478

Differs from previously described *Ellisembia* species in its flask-shaped conidia with a subhyaline multiseptate rostrum.

Type: China. Hainan Province: tropical forest of Bawangling, on decaying branches of *Pistacia chinensis* Bunge (*Anacardiaceae*), 30 October 2011, Sh.C. Ren (**Holotype** HSAUP H8620; **isotype** HMAS 243418).

Etymology: in reference to the host genus, *Pistacia*.

Colonies on natural substrate effuse, brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown, smooth-walled hyphae, 2–4 µm wide. Conidiophores distinct, single or in groups, erect, unbranched, straight or flexuous, cylindrical, brown to dark brown, smooth, 3–8-septate, 70–125 µm long, 4.5–6 µm wide. Conidiogenous cells monoblastic, integrated, terminal, cylindrical, brown, smooth, determinate. Conidial secession schizolytic. Conidia holoblastic, solitary, acrogenous, flask-shaped, rostrate, smooth-walled, brown, rostrum pale brown to subhyaline, 8–10-distoseptate, 50–65 µm long (rostrum included), 13–14.5 µm wide in the broadest part, 3.5–4 µm wide at the truncate base, rostrum 25–40 µm long, 6–8 µm wide.

Comments – *Ellisembia pistaciae* is unique in having determinate conidiogenous cells and flask-shaped conidia with a multiseptate, pale coloured rostrum measuring 25–40 × 6–8 µm.

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Lichenized and lichenicolous fungi new to Babia Góra National Park (Poland, Western Carpathians)

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Abstract — 46 lichenized fungi and 1 lichenicolous fungus new to the Babia Góra Massif, a Polish and Slovak transborder mountain range, the highest in the flysch Western Carpathians, are presented here. Taxonomic and ecological remarks, as well as the distribution of some noteworthy taxa in other Carpathian ranges, are included. *Helocarpon crassipes* is reported in detail for the first time from the Western Beskidy Mts and *Leptogium intermedium* is rediscovered in the Polish Carpathians.

Key words — lichen diversity, lichen biota, lichens, Biosphere Reserve

Introduction

The Babia Góra Massif, the highest mountain range (1725 m a.s.l.) of the Western Beskidy Mts (part of the Western Carpathians), is located on the Polish-Slovak border. The main part of this transborder range belongs to Poland and is covered by the Biosphere Reserve of UNESCO MAB. Since the whole Babia Góra Massif has a unique and valuable natural character, an extension of the Reserve into Slovakia part is planned. The Protected Landscape Area 'Horna Orava' has covered the Slovak portion of the Massif since 1979, while the Polish side is almost entirely included within the Babia Góra National Park since 1954. Different nature conservation methods on both sides of the massif are nonetheless linked by compatible protection regimes.

The natural environment of the Carpathians was extensively exploited throughout the whole of the 18th and early 19th centuries, with mass deforestation and replacement by spruce monocultures on the degraded areas contributing to an impoverished epiphytic lichen biota. Moreover, excessive grazing of large cattle herds in the alpine and subalpine zones caused the destruction of terrestrial lichens, important components of alpine grasslands.

The diversity of plant communities, forming vegetation zones from the lower forest zone to the alpine zone in the Babia Góra Massif, and especially relics of ancient upper spruce montane forests, continues to have a direct impact on the unique character of the lichen biota of the massif, which includes 350 species of both lichenized and lichenicolous fungi (Bielczyk 2004; Czarnota 2007; Kukwa et al. 2010).

The first reports on lichens of the Babia Góra Massif are by Stein (1872, 1873), whose data are repeatedly cited in the summary studies by Rehman (1879) and Boberski (1886). These contain information on some macrolichens (e.g. *Usnea longissima*, *Sticta sylvatica*) no longer recorded in this area, despite intensive lichenological studies were made by Suza (1951), Tobolewski (1966) and Nowak (1998). Other minor works referring mainly to the 'Atlas of the geographical distribution of lichens in Poland' and detailed history of all studies on lichens in the Babia Góra Massif are provided by Bielczyk (2004). In the first decade of this century, the area of Babia Góra NP has once more become a site of lichenological interest, with new reports of lichens from this region (Wegrzyn 2002; 2004). Except for the contributions by Lisická (1980) on two *Umbilicaria* species and Guttova (2001) on *Microcalicium disseminatum*, there are no additional published data from the Slovak side of Babia Góra Massif.

The known lichen diversity of the neighbouring lower flysch ranges of Western Beskidy Mts (Kiszka 1967; Nowak 1998; Czarnota 2010) suggests that the biodiversity is probably much greater in the Babia Góra Massif than published; indeed, our current lichenological results significantly extend the list by 47 taxa. However, we did not make a detailed exploration of all available lichen habitats in 2007–10 but focused on lichens occupying standing and fallen spruce 'dead wood' (including wind-throws) within the strictly protected area and investigated lichen diversity within 520 permanent plots regularly distributed throughout the park, including the highest alpine zone.

Materials & methods

All collections except one were made in Babia Góra National Park. Lichens were identified by standard microscopy, with *Lepraria*, *Cladonia* and some sterile sorediate species checked through thin-layer chromatography (TLC) in solvent C according to Orange et al. (2001). Apothecial hand-made sections were mounted in water and anatomical structures were measured in KOH. Spot test reactions of apothecial pigments were studied in KOH and NaClO, and ascus types as well as medulla in IKI. Nomenclature follows MycoBank Fungal Databases (http://www.mycobank.org).

Examined materials and selected habitat notes are given for all taxa, while distributional, ecological and taxonomic notes are included for more noteworthy and rare species. The P. Czarnota collections are deposited in the Gorce National Park (GPN) herbarium while M. Węgrzyn collections are in the Jagiellonian University herbarium in Kraków (KRA).

Abbreviations: fs no. = forest section number; * = lichenicolous fungus.

The species

Absconditella delutula (Nyl.) Coppins & H. Kilias

Specimens examined: **POLAND. Małopolska Province**, SE slope of Kępa hill, fs no. 25c [49°34′29.4″N 19°33′41.3″E], alt. 1360 m, 26.8.2009, leg. P. Czarnota 6339 (GPN); E slope of Sokolica hill, fs no. 17b [49°35′15.1″N 19°34′26.2″E], alt. 1140 m, 9.6.2009, leg. P. Czarnota 6046 (GPN); NE slope of Sokolica hill fs no. 5b close to 'Dolny Płaj' tourist track [49°35′26.8″N 19°34′14.9″E], alt. 1040 m, 10.6.2009, leg. P. Czarnota 5991 (GPN); 'Górny Płaj' tourist track, near Mokry Staw lake, fs no. 18b [49°35′25.18″N 19°33′57.2″E], alt. 1040 m, 10.11.2010, leg. M. Węgrzyn 4153 (KRA).

Ecology & distribution: Based on both the present study and previous investigations in other Polish ranges (Czarnota unpubl.), *A. delutula* seems not infrequent on sandstone pebbles over root systems of recent conifer wind-throws in montane forests. Sometimes it occurs on decaying wood (Pišút et al. 2007; Malíček et al. 2010).

This ephemeral species has been found several times in Poland (Czarnota & Kukwa 2008a). The new sites extend its range in the Western Carpathians where it has previously been reported from both Polish (Gorce, Beskid Wyspowy Mts) and Czech (Moravskoslezké Beskydy) areas (Czarnota & Kukwa 2008a; Malíček et al. 2010).

Anisomeridium polypori (Ellis & Everh.) M.E. Barr

Specimen examined: **POLAND, Małopolska Province**, N of Markowe Szczawiny shelter house, fs no. 74b [49°35′48.7″N / 19°31′12.6″E], alt. 880 m, on decaying bark of roots of fallen *Picea abies* within Carpathian beech forest, 2.7.2009, leg. P. Czarnota 6120 (GPN).

Biatora chrysantha (Zahlbr.) Printzen

Specimens examined: **POLAND. Małopolska Province**, N slope of massif, near Rybny Potok stream, fs no. 59a [49°36′53.2″N / 19°32′15.3″E], alt. 750 m, on *Alnus glutinosa* bark, 4.8.2010, leg. M. Węgrzyn 4214 (KRA); NE slope of Sokolica, fs no. 17b [49°35′15.0″N / 19°34′17.09″E], alt. 1200 m, on *Sorbus aucuparia* bark, 9.6.2009, leg. M. Węgrzyn 3169 (KRA).

Ecology & distribution: *B. chrysantha* prefers old, natural forests, where it is found at the base of older trees and on over-growing bryophytes in high precipitation sites.

This species has been found in Poland several times in the Carpathians (Fałtynowicz 2003) and Western Pomerania (Kukwa & Szymczyk 2006). Close to Babia Góra Massif it was reported only from Wielka Racza Mt. (Nowak 1998). In Europe its distribution is defined as suboceanic, since it is widely distributed in Scandinavia, Finland and the British Isles (Printzen & Palice 1999), but it is also recorded in the central, mountainous part of Europe (e.g. Coppins et al. 1998, 2005; Kiszka & Kościelniak 1998; Printzen & Palice 1999; Pišút et al. 2007).

Biatora helvola Körb. ex Hellb.

Specimen examined: E slope of Sokolica hill, fs no. 25a [49°35′08.8″N / 19°34′05.9″E], alt. 1280 m, on *Sorbus aucuparia* bark within upper montane spruce forest, 9.6.2009, leg. P. Czarnota 6026 (GPN).

Biatora veteranorum Coppins & Sérus. Plate 1a

≡ *Catillaria alba* Coppins & Vězda

This species is almost exclusively cited in the literature as *Catillaria alba* (Palice 1999; Fletcher & Coppins 2009), since it has only recently been

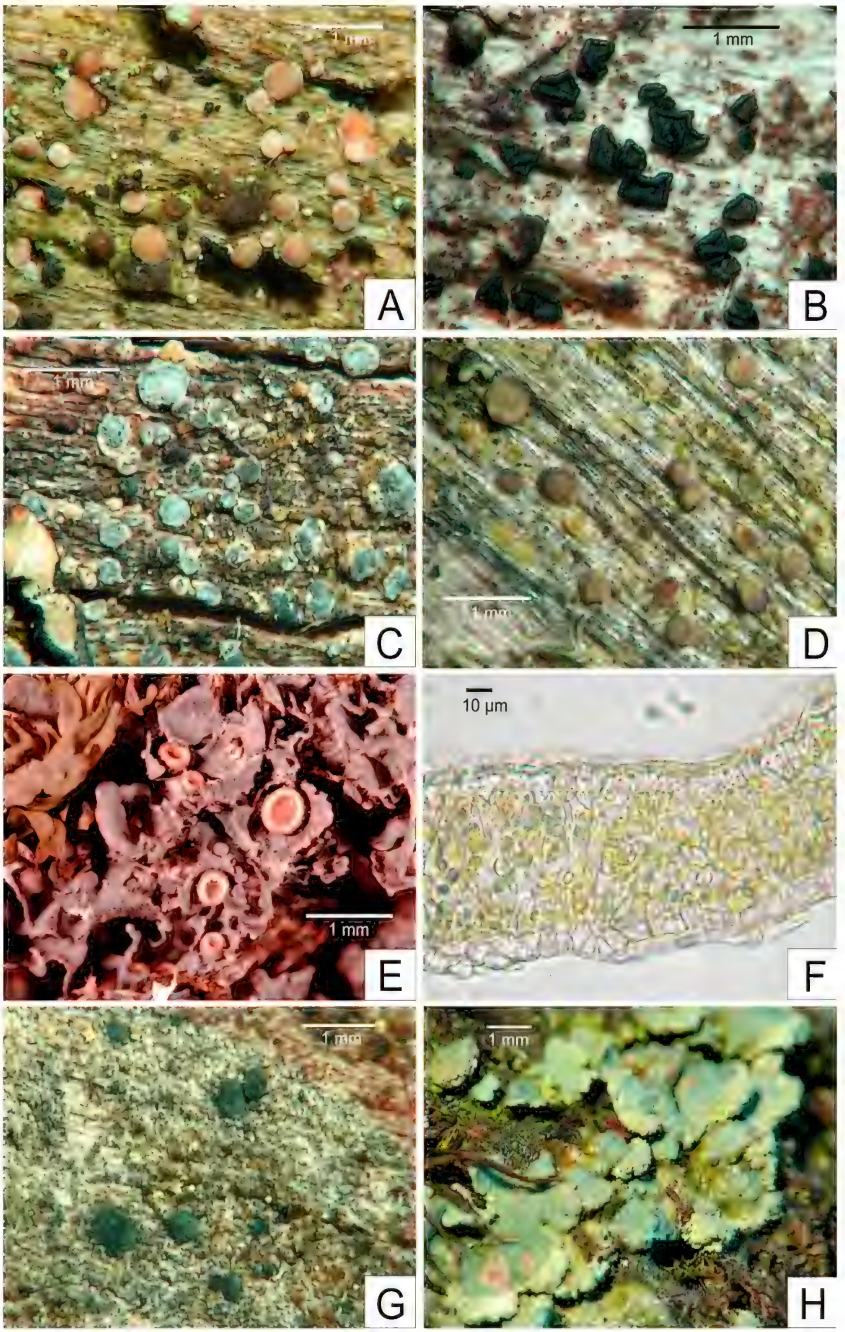
transferred to *Biatora* (as *B. veteranorum* nom. nov., non *B. alba* Hepp) on the basis of its phylogenetic affinity to several other *Biatora* taxa (Sérusiaux et al. 2010). Vězda (1993) originally noted in the protologue that as this species does not belong to the genus *Catillaria* sensu stricto, the name *Catillaria alba* should be treated rather tentatively. Indeed, the 1-septate, ellipsoid ascospores, ascus tholus with a darker tube and non-amyloid conical ocular chamber, unpigmented apical caps of paraphyses, small bacilliform conidia, and the usual presence of short-stalked pycnidia suggest that the species may belong to another genus within, for example, *Pilocarpaceae* (Sérusiaux et al. 2010). Such pycnidia are almost unknown in *Biatora* except for *B. ligni-mollis* T. Sprib. & Printzen, which forms rather sessile, globose, and never white pruinose pycnidia, whereas in *B. veteranorum* they are distinctly stalked, elongated, and almost white from distinct pruina. The 'core' pycnidial wall in *B. veteranorum* is white to pale beige, contrasting with the orange or slightly brown pycnidial pigmentation in *B. ligni-mollis*. Such pycnidial characters of major diagnostic importance for *B. veteranorum,* which is often found in only in the asexual stage. Stalked, pale pycnidia are also present in several *Micarea* species, e.g., *M. doliiformis* (Coppins & P. James) Coppins & Sérus., *M. hedlundii* Coppins, *M. pycnidiophora* Coppins & P. James, *M. stipitata* Coppins & P. James, and *M. tomentosa* Czarnota & Coppins (Coppins 1983; Czarnota 2007; Sérusiaux et al. 2010), but there they are at most whitish tomentose and never pruinose. Moreover, all these species differ from *B. veteranorum* in their external thallus. All *B. veteranorum* specimens from the Babia Góra Massif have abundant apothecia, which are also white pruinose due to minute crystals confined to the epithecium.

Specimens examined: **POLAND. Małopolska Province**, N slope of Diablak Mt., fs no. 23a [49°34′55″N / 19°31′10.7″E], alt. 1270 m, 7.8.2009, leg. P. Czarnota 6265 (GPN); N slope of Sokolica hill, fs no. 7a [49°35′21.0″N / 19°33′12.9″E], alt. 1060 m, 21.7.2009, leg. P. Czarnota 6225 (GPN); N slope of Diablak Mt., fs no. 10g [49°34′57.3″N / 19°31′51.6″E], alt. 1180 m, 3.7.2009, leg. P. Czarnota 6172 (GPN). **POLAND. Podkarpackie Province**, Bieszczady Mts, Bieszczadzki National Park, Sianki forest district, fs no. 78i, on wood of a decaying *Abies alba* stump, mixed forest dominated by *Picea abies*, 19.6.2002, leg. P. Czarnota 2872 (GPN).

Ecology & distribution: *B. veteranorum* prefers slightly decayed wood of decorticated higher conifer snags within dense mixed and spruce montane forests, especially in lower zones, where it usually occupies shady niches.

This species has previously only been reported from Poland twice, both from the Polish Western Carpathians (Czarnota 2003, 2010; as *Catillaria alba*). The new records

Plate. 1. Habits of several noteworthy species. A – *Biatora veteranorum* [Czarnota 6225 (GPN)]; B – *Elixia flexella* [Czarnota 6266 (GPN)]; C – *Lecanora sarcopidoides* [Czarnota 4199 (GPN)]; D – *Lecanora subintricata* [Czarnota 6062 (GPN); E – *Leptogium intermedium* [Węgrzyn 4151 (KRA)]; F – cross section of thallus of *Leptogium intermedium* [Węgrzyn 4151 (KRA)]; G – *Lecidea leprarioides* [Czarnota 6177 (GPN)]; H – *Trapeliopsis glaucolepidea* [Czarnota 6012 (GPN)].


1 mm
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from Babia Góra Massif and Bieszczady Mts in Eastern Carpathians extend its range to the entire Polish part of this mountain range. *B. veteranorum* was also recorded in the Ukrainian Carpathians (Kondratyuk & Coppins 2000).

Calicium glaucellum Ach.

Specimens examined: **POLAND. Małopolska Province**, N slope of Sokolica hill fs no. 7a [49°35′21.0″N / 19°33′12.9″E], alt. 1060 m, 21.7.2009, leg. P. Czarnota 6226 (GPN); ibid., [49°35′25.9″N / 19°33′12.2″E], alt. 1055 m, 21.7.2009, leg. P. Czarnota 6235 (GPN); ibid., fs no. 24a, below Brona Pass [49°35′07.3″N / 19°30′41.4″E], alt. 1250 m, 21.8.2009, leg. P. Czarnota 6311 (GPN); S slope of Kępa hill, fs no. 26h [49°34′07.8″N / 19°33′12.8″E], alt. 1290 m, 17.7.2009, leg. P. Czarnota 6078 (GPN); N slope of Diablak Mt., fs no. 10g [49°34′58.2″N / 19°31′45.7″E], alt. 1180 m, 3.7.2009, leg. P. Czarnota 6140 (GPN); N slope of Babia Góra Massif, Czatoża Forest, fs no. 16a [49°35′42.0″N / 19°28′49.7″E], alt. 1200 m, 23.6.2009, leg. M. Węgrzyn 3792 (KRA).

Ecology & distribution: Similar to several other *Calicium* species, *C. glaucellum* is found on decaying wood of decorticated conifer trunks within different montane forests up to the timber line.

Although Tobolewski (1966) focused on species of the former *Caliciaceae* during his explorations of Babia Góra, he did not report this currently quite common epixylic lichenized fungus. However, in the Tobolewski key to *Calicium* (Nowak & Tobolewski 1975), it was likely to be confused with *C. abietinum* Pers.

Candelariella aurella (Hoffm.) Zahlbr.

Specimen examined: **POLAND. Małopolska Province**, S slope of Diablak Mt., above the Rajsztag road, fs no. 99h [49°33′45.1″N / 19°43′46.8″E], alt. 905 m, on concrete of old building ruins, 11.6.2008, leg. M. Węgrzyn 4106 (KRA).

Chaenothecopsis nigra Tibell

Specimens examined: **POLAND. Małopolska Province**, N slope of Diablak Mt., fs no. 10g [49°34′56.3″N / 19°31′55.2″E], alt. 1170 m, 3.7.2009, leg. P. Czarnota 6133 & 6134 (GPN); ibid., [49°34′58.0″N / 19°31′52.1″E], alt. 1170 m, 3.7.2009, leg. P. Czarnota 6161 (GPN); below Brona Pass, fs no. 24a, [49°35′10.1″N / 19°30′47.1″E], alt. 1240 m, 14.7.2009, leg. P. Czarnota 6188 (GPN).

Ecology & distribution: Most specimens were collected on free-living algae *Stichococcus* in association with *Chaenotheca stemonea* on bark of dead spruces at trunk bases, but it was also found on *Stichococcus* over soil in root-system underhangs of fallen *Picea abies*; all collections were within upper montane spruce forest.

This species is often included in lists of lichenicolous fungi (e.g. Bielczyk 2003), but it is also sometimes lichenized (Giavarini & Coppins 2009) as in this case. From Poland *C. nigra* has been reported only twice (Fałtynowicz 2003), including a record from Western Beskidy Mts (Bielczyk 2003) lacking details.

Cladonia cenotea (Ach.) Schaer.

Specimens examined: **POLAND. Małopolska Province**, S slope of Kępa hill, fs no. 26h [49°34′09.3″N / 19°33′03.2″E], alt. 1365 m, 17.7.2009, leg. P. Czarnota 6085 (GPN); N slope of the massif below Brona pass, fs no. 24a [49°35′01.0″N / 19°30′33.5″E], alt. 1330 m, 3.7.2010, leg. M. Węgrzyn 4005 (KRA).

Ecology & distribution: Common in the Polish Carpathians (Fałtynowicz 2003; Bielczyk 2003), this species is found on decaying wood of roots, soil, and plant debris in upper montane spruce forests.

Cladonia gracilis (L.) Willd.

Specimens examined: **POLAND. Małopolska Province**, S slope of Gówniak hill, Wolarnia, fs no. 28f [49°34′14.3″N / 19°32′13.0″E], alt. 1575 m, on soil, 10.7.2010, leg. M. Węgrzyn 4171 (KRA); close to Gówniak hill, fs no. 21f [49°34′32.18″N / 19°32′27.24″E], alt. 1620 m, on soil, 27.8.2009, leg. M. Węgrzyn 3286 (KRA).

Ecology & distribution: This very common species in lowland Scots pine forests is also recorded in subalpine and alpine zones of mountain regions in Europe.

In the Tatra Mts alpine meadows are dominated by *C. gracilis* and *C. amaurocraea* (Flörke) Schaer. is rare (Osyczka & Węgrzyn 2005), but the situation is reversed in the Babia Góra Massif (Węgrzyn unpubl.). Because *C. amaurocraea* and *C. macroceras* (Delise) Ahti are morphologically very similar to *C. gracilis* in this region, species determination requires more attention, including investigation of their secondary metabolites (Osyczka & Węgrzyn 2005).

Cladonia grayi G. Merr. ex Sandst.

Specimen examined: **POLAND. Małopolska Province**, S of Sokolica hill, fs no. 25c [49°34′58.3″N / 19°33′48.3″E], alt. 1290 m, on humus over root system of fallen *Picea abies* within upper montane spruce forest, 1.7.2009, leg. P. Czarnota 6114 (GPN).

Cladonia norvegica Tønsberg & Holien

Specimen examined: **POLAND. Małopolska Province**, E slope of Sokolica hill, fs no. 25a [49°35′07.9″N / 19°34′06.4″E], alt. 1290 m, on roots of fallen *Picea abies* within upper montane spruce forest, 9.6.2009, leg. P. Czarnota 6011 (GPN).

Distribution: The species is rare in Poland. Osyczka (2011) confirms its presence in the Tatra Mts, the Pieniny Mts, and the region of the Puszcza (Primeval) Augustowska and Puszcza Knyszyńska forests in northeast Poland.

Cladonia ochrochlora Flörke

The encountered specimen consists of several subulate and scyphose podetia. Generally, *C. ochrochlora* is characterized by areolate cortication with cortex especially well developed basally but sometimes extending upwards to near the apices or even inside narrow scyphi. The surface without cortex is covered by farinose to granular soredia (e.g. Ahti 2000; James 2009). This description can be applied to the specimen examined; however, some of the gathered podetia are almost totally areolate-corticate, partly richly squamulose, and produce only scattered granulose soredia. The specimen was fertile, with most of the podetia terminating in pale brown contiguous apothecia.

Specimen examined: **POLAND. Małopolska Province**, N foot of Diablak Mt., Stonów, fs no. 50b [49°36′21.1″N / 19°33′3.8″E], alt. 770 m, on decaying wood, 21.5.2010, leg. M. Węgrzyn 4008 (KRA).

Ecology & distribution: This species has previously been rather frequently reported from the Polish Carpathians (see Bielczyk 2003), but not from the Babia Góra Massif. The specimen was overgrowing decayed spruce wood.

Diploschistes muscorum (Scop.) R. Sant.

Specimen examined: **POLAND. Małopolska Province**, the main ridge of massif, close to Gówniak hill, fs no. 21f [49°34′27.84″N / 19°32′12.99″E], alt. 1680 m, on base-rich soil between calcareous sandstones, 27.8.2009, leg. M. Węgrzyn 4215 (KRA).

Elixia flexella (Ach.) Lumbsch PLATE 1B

SPECIMENS EXAMINED: **POLAND. MAŁOPOLSKA PROVINCE**, N slope of Diablak Mt., close to Suchy Stawek, fs no. 23a [49°34′55″N / 19°31′10.7″E], alt. 1270 m, leg. P. Czarnota 6266 (GPN); SE slope of Kępa hill, fs no. 25c [49°34′20.9″N / 19°33′52.9″E], alt. 1200 m, 17.7.2009, leg. P. Czarnota 6063 (GPN); below Brona Pass, fs no. 24a [49°35′09.3″N / 19°30′51.6″E], 1235 m, 14.7.2009, leg. P. Czarnota 6195 (GPN); N slope of Diablak Mt., fs no. 10g [49°34′58.2″N / 19°31′45.7″E], alt. 1180 m, 3.7.2009, leg. P. Czarnota 6141 (GPN).

ECOLOGY & DISTRIBUTION: Quite common on decaying wood of decorticated *Picea abies* trunks and fallen spruce roots within the upper montane spruce forest, *E flexella* is known elsewhere in the Polish Western Carpathians (Bielczyk 2003).

****Endococcus propinquus*** (Körb.) D. Hawksw.

SPECIMENS EXAMINED (both on sterile epilithic lichens): **POLAND. MAŁOPOLSKA PROVINCE**, SE slope of Kępa hill, fs no. 25c [49°34′21.8″N / 19°33′52.0″E], alt. 1210 m, 17.7.2009, leg. P. Czarnota 6072 (GPN); N slope of Sokolica hill, fs no. 18b [49°35′26.5″N / 19°34′02.4″E], alt. 1050 m, 10.6.2009, leg. P. Czarnota 6049 (GPN).

ECOLOGY & DISTRIBUTION: This lichenicolous fungus is probably common in the Babia Góra Massif; it grows on various epilithic lichens over sandstone rocks above the timberline but is also found within montane forests.

Fuscidea pusilla Tønsberg

Since Tønsberg (1992) described this species from Scandinavia, its distribution has markedly increased in Europe, despite its being found only in a sterile, usually overlooked or hardly distinguishable, form. The presence of divaricatic acid as the major diagnostic secondary substance appears sufficient for distinguishing *F. pusilla* from several other morphologically similar species: *Ropalospora viridis* (Tønsberg) Tønsberg, *Mycoblastus fucatus* (Stirt.) Zahlbr., *Fuscidea arboricola* Coppins & Tønsberg, and *Buellia griseovirens* (Turner & Borrer ex Sm.) Almb. *Ropalospora viridis,* also sterile (except a few fertile reports; Tønsberg 1992), differs almost only in the presence of perlatolic acid and thus is not UV+ bluish-white (contrasting with *F. pusilla*, which contains divaricatic acid). The other three species, which also contain substances other than divaricatic acid, are easily detected in simple spot tests such as: P+ ginger from fumarprotocetraric acid (*M. fucatus* and *F. arboricola*) and K+ yellow turning to brownish-red and P+ yellow to yellow-orange due to norstictic acid and substances of stictic acid complex in *B. griseovirens*. (Sometimes this K+ reaction is almost invisible, as the norstictic acid concentration is below the detection limit, but then characteristic norstictic acid crystals can be observed in the slide preparation.) Lendemer (2011) provides excellent morphological and chemical comparisons of *F. pusilla* and *R. viridis* supported by colour figures.

SPECIMENS EXAMINED (selected): **POLAND. MAŁOPOLSKA PROVINCE**, N slope of Diablak Mt., fs no. 22a [49°34′51.3″N / 19°31′41.6″E], alt. 1220 m, 7.8.2009, leg. P.

Czarnota 6273 (GPN); E slope of Sokolica hill, fs no. 25a [49°35′07.9″N / 19°34′06.4″E], alt. 1290 m, 9.6.2009, leg. P. Czarnota 6010 (GPN); N slope of Diablak Mt., fs no. 10g [49°34′56.3″N / 19°31′55.2″E], alt. 1170 m, 3.7.2009, leg. P. Czarnota 6165 (GPN); E slope of Sokolica hill, fs no. 25a [49°35′08.2″N / 19°34′07.4″E], alt. 1280 m, 9.6.2009, leg. P. Czarnota 6201 (GPN); S slope of Kępa hill, fs no. 26h [49°34′07.8″N / 19°33′02.5″E], alt. 1365 m, 17.7.2009, leg. P. Czarnota 6214 (GPN); S of Sokolica hill, fs no. 25c [49°35′01″N / 19°33′47.6″E], alt. 1285 m, 1.7.2009, leg. P. Czarnota 6104 (GPN).

ECOLOGY & DISTRIBUTION: *Fuscidia pusilla* has rarely been reported (normally as an epiphyte of deciduous trees) from the Polish and Ukrainian Carpathians (Bielczyk 2003; Kościelniak & Kiszka 2003; Kondratyuk & Coppins 2000), but in the Babia Góra Massif it is found as a common lichenized fungus on wood of dead decorticated spruces, snags and wind-throws.

The species has not been reported from the British Isles (Gilbert et al. 2009), whereas another divaricatic acid-producing sorediate *Fuscidia*, *F. lightfootii* (Sm.) Coppins & P. James, is well known there. An assumption that both species may represent the same taxon and that *F. pusilla* is only a morph of *F. lightfootii* (Gilbert et al. 2009) has no molecular support. A mtSSU rDNA phylogeny of *Fuscideaceae* shows that the two species are not closely related (Bylin et al. 2007).

Helocarpon crassipes Th. Fr.

≡ *Micarea crassipes* (Th. Fr.) Coppins

Although molecular evidence suggests that *H. crassipes* is better treated in the separate genus *Helocarpon* Th. Fr. (Andersen & Ekman 2005), it is still sometimes placed in *Micarea* Fr. (Coppins 2009). Indeed, the 'micareoid' photobiont, sparingly branched and anastomosed paraphyses, ascus structure, and mixture of Cinereorufa-green, Melaena-red, and Melaenida-red apothecial pigments (Meyer & Printzen 2000) are phenotypic features found in some representatives of *Micarea* s. lat. (Coppins 1983). *Micarea assimilata* (Nyl.) Coppins in particular resembles *H. crassipes* in apothecial pigmentation, lack of secondary substances, and ecology. Both species are terricolous and have an arctic-alpine distribution (Coppins 1983), but they differ at least in the thallus and apothecial structure. *Micarea assimilata* forms thick, slightly convex areoles intermixed with cephalodia of its additional photobiont, *Nostoc*, while the *H. crassipes* thallus is composed of small, ± globose, often coralloid areoles, with only a chlorococcoid photobiont. Young *H. crassipes* apothecia are marginate and constricted below hypothecium forming the distinct stalked base in contrast to the *M. assimilata* apothecia, which are never marginate and shortly stalked. Sterile forms of *H. crassipes* markedly resemble thalli of *M. leprosula* and *M. submilliaria* (Nyl.) Coppins and can grow together in alpine regions. However, both these species produce easily detected metabolites, namely gyrophoric acid and argopsin in *M. leprosula* (C+ red, Pd+ ginger) and alectorialic acid in *M. submilliaria* (Pd+ yellow), and their thallus areoles are very fragile. Furthermore, both species differ from *H. crassipes* in their apothecial characters and spore septation (for more details see Czarnota 2007). Although *Micarea* appears not to be monophyletic (Andersen & Ekman 2005)

and phenetically not congeneric with *Helocarpon, H. crassipes* may represent *Micarea* in its classical sense by Coppins (1983), as many other representatives are morphologically distantly related to the 'core' *Micarea prasina* group. However, in the mtSSU sequence-based phylogenetic tree of *Lecanorales, H. crassipes* nests outside the former *Micareaceae*. We therefore prefer to retain the species in the separate genus *Helocarpon*.

Specimen examined: **POLAND. Małopolska Province**, Gówniak hill, by tourist track [49°34′34″N / 19°32′39″E], alt. 1608 m, on soil and decaying bryophytes, 11.11.2005, leg. P. Czarnota 4600 (GPN).

Ecology & distribution: This terricolous species occurs frequently in the alpine zone of the Tatra Mts (Bielczyk 2003; Czarnota unpubl.). Its presence in the Babia Góra Massif was noted by Czarnota (2007), but without locality details. Formerly *Helocarpon crassipes* was often recognized as *Lecidea assimilata,* under which name it is filed in some Polish herbaria (see Czarnota 2007).

Lecanora chlarotera Nyl.

Specimens examined (selected; each on a different deciduous tree): **POLAND. Małopolska Province**, S slope of Diablak Mt., near the Pl–Sk boundary [49°33′23.47″N / 19°31′47.59″E], alt. 1200 m, 14.5.2008, leg. M. Węgrzyn 3844 (KRA); Zawoja Markowa, fs no. 72a [49°36′14.2″N / 19°31′13.0″E], alt. 740 m, 11.7.2010, leg. M. Węgrzyn 4159 (KRA); N slope of massif, W of Sulowa Cyrhla glad, fs no. 71a [49°35′49.4″N / 19°31′36.9″E], alt. 870 m, 5.8.2010, leg. M. Węgrzyn 4160 (KRA).

Lecanora expallens Ach.

Specimens examined (selected): **POLAND. Małopolska Province**, N slope of Diablak Mt., fs no. 23a, close to Suchy Stawek [49°34′55″N / 19°31′10.7″E], alt. 1270 m, 7.8.2009, leg. P. Czarnota 6268 (GPN); N slope of Sokolica hill, fs no. 7a [49°35′25.6″N / 19°33′06.9″E], alt. 1055 m, 25.8.2009, leg. P. Czarnota 6328 (GPN); S slope of Kępa hill, fs no. 26h [49°34′09.3″N / 19°33′09.2″E], alt. 1315 m, 17.7.2009, leg. P. Czarnota 6060 (GPN); ibid., [49°34′07.8″N / 19°33′12.8″E], alt. 1290 m, 17.7.2009, leg. P. Czarnota 6080 (GPN).

Ecology & distribution: This usually sterile species is frequently reported from the Polish Carpathians (Bielczyk 2003) primarily as an epiphyte on deciduous trees. In Babia Góra Massif as in other higher ranges of the Western Carpathians, however, it frequently grows also on hard wood of decorticated dead spruces within upper montane forests (Czarnota unpubl.).

Lecanora saligna (Schrad.) Zahlbr. var. *saligna*

Specimens examined (on decaying wood, bark of snags and roots of fallen spruces): **POLAND. Małopolska Province**, N of Markowe Szczawiny shelter house, fs no. 74b [49°35′48.7″N / 19°31′12.6″E], alt. 880 m, 2.7.2009, leg. P. Czarnota 6121 (GPN); below Brona Pass, fs no. 24a, [49°35′44.0″N / 19°31′10.3″E], alt. 940 m, 2.7.2009, leg. P. Czarnota 6131 (GPN); SE slope of Kępa hill near the Chodnik Parkowy trail, fs no. 27f [49°34′04.2″N / 19°33′02.1″E], alt. 1350 m, 28.5.2009, leg. M. Węgrzyn 3235 (KRA).

Lecanora sarcopidoides (A. Massal.) Hedl. Plate 1c

Specimen examined: **POLAND. Małopolska Province**, below Brona Pass, fs no. 24a [49°35′10.8″N / 19°30′45.7″E], alt. 1240 m, 14.7.2009, leg. P. Czarnota 6194 (GPN).

Lecanora subintricata (Nyl.) Th. Fr. PLATE 1D

This species usually develops an endoxylic thallus (Edwards et al. 2009), but occasionally a form with a thick, warted, slightly yellowish thallus is encountered as elsewhere in the Polish Carpathians (Czarnota 2010). It differs from other morphologically similar *Lecanora* species (e.g. *L. sarcopidoides*, *L. saligna*, endoxylic forms of *L. conizaeoides* Nyl. ex Cromb., *L. albellula* (Nyl.) Th. Fr.) in its chemistry (presence of only usnic acid) and smaller ascospores. Apothecia of *L. subintricata* are variously shaped and pigmented, and the presence of a more or less developed amphithecium depends on their ontogeny and ecological conditions. They are mostly concave, olive-grey and slightly yellowish-green at least in the margin, but sometimes apothecia of *L. subintricata* in similar circumstances are olive-brown to brown and often convex and immarginate, resembling those found in *L. saligna* var. *saligna* or some forms of *L. phaeostigma* (Körb.) Almb. Some apothecia can also be slightly pruinose, as in *L. sarcopidoides*, which, however, has larger ascospores and pseudoplacodiolic acid as the major substance. *Lecanora conizaeoides* is easily distinguished by P+ ginger reaction (fumarprotocetraric acid) in its apothecial margin. The presence of brialmontin 1 as an additional minor secondary compound (Edwards et al. 2009) was not detected in the tested Polish *L. subintricata* specimens.

SPECIMENS EXAMINED (selected): **POLAND. MAŁOPOLSKA PROVINCE**, N slope of Sokolica, fs no. 18c [49°35′15.3″N / 19°33′49.7″E], alt. 120 m, 26.8.2009, leg. P. Czarnota 6335 (GPN); below Brona Pass, fs no. 24a, [49°35′06.6″N / 19°30′43.4″E], alt. 1260 m, 21.8.2009, leg. P. Czarnota 6284 (GPN); SE slope of Kępa hill, fs no. 25c [49°34′21.7″N / 19°33′51.9″E], alt. 1210 m, 17.7.2009, leg. P. Czarnota 6081 (GPN); S slope of Kępa hill, fs no. 26h [49°34′07.7″N / 19°33′01.9″E], alt. 1365 m, 17.7.2009, leg. P. Czarnota 6067 (GPN).

ECOLOGY & DISTRIBUTION: First modern records of *L. subintricata* for Poland were recently confirmed in the Gorce Mts (Czarnota 2010). Both there and in the Babia Góra Massif, *L. subintricata* is very common, mostly occupying decayed decorticated spruce wood, especially frequent in the upper spruce forests much destroyed by the activity of the bark beetle *Ips typographus*. In the Polish Western Carpathians, *L. subintricata* prefers hard wood exposed to the light and with *L. expallens*, *L. phaeostigma*, non-lichenized *Mycocalicium subtile* (Pers.) Szatala, and *Strangospora moriformis* appears to be one of the first common colonizers of this substrate.

Lecidea leprarioides Tønsberg PLATE 1G

This species, which recently was reported from Gorce Mts as new for Poland (Czarnota 2010), was collected previously in other Carpathian regions as *Lecidea turgidula* Fr. or *L. turgidula* var. *pulveracea* Th. Fr. (Czarnota unpubl.). Indeed, *L. leprarioides* and *L. turgidula* often have almost identical apothecia, especially when the thallus of *L. leprarioides* is less developed and the *L. turgidula* apothecia are whitish to bluish pruinose. In such cases secondary compound analyses help differentiate the usually inconspicuously endosubstratal *L. turgidula* (which produces placodiolic acid) and distinctly sorediate *L. leprarioides* (which

contains pseudoplacodiolic acid, rarely present in other lichens; Tønsberg 1992). Similar sorediate thalli, often found in similar habitats in the Carpathians, may also form in *L. nylanderi* (Anzi) Th. Fr. and diminutive examples of *Ochrolechia microstictoides* Räsänen. However, both species are usually sterile, in contrast to the usually fertile *L. leprarioides*, which is easily distinguished by its immarginate, convex, bluish-grey apothecia immersed within sorediate patches. All species differ markedly in their chemistry: *L. nylanderi* contains divaricatic acid and *O. microstictoides* variolaric and lichesterinic acids. Furthermore, their soredia are thicker and more granular, and thus their thalli are less farinose than the thallus of *L. leprarioides*. Another similar, usually sterile, sorediate epiphyte growing on acidic bark, which also sometimes serves as substrate for *L. leprarioides*, is *Cliostomum leprosum* (Räsänen) Holien & Tønsberg. This species is, however, rare (reported mainly from N Europe; Golubkov & Kukwa 2006) and its major compounds are atranorin and caperatic acid (Tønsberg 1992). For an excellent description of *L. leprarioides* and similarities to other morphologically related taxa, see also Tønsberg (1992) and Aptroot et al. (2009).

Specimens examined (selected): **POLAND. Małopolska Province**, N slope of Sokolica hill, fs no. 18c [49°35′13.7″N / 19°33′48.3″E], alt. 1200 m, 26.8.2009, leg. P. Czarnota 6302 (GPN); N slope of Diablak Mt., fs no. 21b [49°35′04.6″N / 19°32′15.3″E], alt. 1160 m, 3.7.2009, leg. P. Czarnota 6156 (GPN); below Brona Pass, fs no. 24a [49°35′10.2″N / 19°30′50.4″E], alt. 1230 m, 14.7.2009, leg. P. Czarnota 6177 (GPN); SE slope of Sokolica hill near the Chodnik Parkowy trail, fs no. 25c [49°34′44.0″N / 19°33′51.5″E], alt. 1380 m, 24.5.2009, leg. M. Węgrzyn 3823 (KRA); ibid., [49°34′51.7″N / 19°34′06.5″E], alt. 1300 m, 24.5.2009, leg. M. Węgrzyn 4152 (KRA).

Ecology & distribution: *Lecidea leprarioides* is a common lichenized fungus usually found on decayed wood of conifer snags, but sometimes it also grows directly on bark of spruces, more particularly within destroyed parts of upper montane spruce forests, similar to *Lecanora subintricata*.

Lepraria eburnea J.R. Laundon

Specimens examined (selected): **POLAND. Małopolska Province**, N slope of Sokolica hill, fs no. 18c [49°35′13.7″N / 19°33′48.5″E], alt. 1200 m, 26.8.2009, leg. P. Czarnota 6349 (GPN); ibid., fs no. 6d [49°35′34.6″N / 19°33′34.5″E], alt. 990 m, 25.8.2009, leg. P. Czarnota 6319 (GPN); E slope of Kępa hill, fs no. 25c [49°34′21.8″N / 19°33′52.0″E], alt. 1210 m, 17.7.2009, leg. P. Czarnota 6073 (GPN).

Ecology & distribution: On tree bark, decaying wood, and sandstone rocks in the lower and upper zones of Babia Góra Massif.

Lepraria jackii Tønsberg

This species is differentiated from other *Lepraria species* by an almost mealy thallus that is shared only by *L. toensbergiana,* from which it differs only chemically (Bayerová et al. 2005).

Specimens examined (selected): **POLAND. Małopolska Province**, N slope of Diablak Mt., fs no. 22a [49°34′51.3″N / 19°31′41.6″E], alt. 1220 m, 7.8.2009, leg. P. Czarnota 6272 (GPN); E slope of Sokolica hill, fs no. 25a [49°35′08.8″N / 19°34′05.9″E],

alt. 1280 m, 9.6.2009, leg. P. Czarnota 6053 (GPN); ibid., fs no. 18c [49°35′7.4″N / 19°33′52.5″E], alt. 1350 m, 6.6.2009, leg. M. Węgrzyn 3261 (KRA); SE slope of Kępa hill, fs no. 25c [49°34′20.9″N / 19°33′52.9″E], alt. 1200 m, 17.7.2009, leg. P. Czarnota 6064 (GPN); N slope of massif, below Dolny Płaj forest road, fs no. 57g [49°35′39.3″N / 19°32′51.6″E], alt. 900 m, 8.5.2010, leg. M. Węgrzyn 4146 (KRA).

Ecology & distribution: This anamorphic lichenized fungus seems to be the most common *Lepraria* in the Western Carpathians. In addition to the Babia Góra Massif, many sites occur in the Gorce Mts (Czarnota & Kukwa 2001), Beskid Sądecki Mts (Kukwa & Śliwa 2005), and Tatra Mts (Kukwa 2004) where it usually grows on conifer bark, decaying stumps and snags, and roots of fallen trees.

Lepraria rigidula (B. de Lesd.) Tønsberg

Specimen examined: **POLAND. Małopolska Province**, N slope Diablak Mt., Sulowa Cyrhla glade [49°36′04.9″N / 19°32′14.4″E], alt. 840 m, on bark of *Salix* sp., 3.5.2010, leg. M. Węgrzyn 4156 (KRA).

Ecology & distribution: Although rather common in Poland (Kukwa 2006), *L. rigidula* has been thus far recorded only from one locality in the Babia Góra Massif. It may be more frequent here, especially on the bark of solitary or roadside deciduous trees at foot of the massif or in old orchards, as data suggest from other West Carpathian ranges (e.g. Kukwa & Śliwa 2005).

Leptogium intermedium (Arnold) Arnold Plate 1e, f

This cyanolichen from the study area has a small thallus composed of numerous spreading squamules up to 3 mm in width, but most do not exceed 1.5 mm. Vertical sections of the thallus are distinctly heteromerous, consisting of dense single-layered zones of large (5–10 μm) cells in the upper and lower cortex surrounding loosely interwoven fungal hyphae in the medulla. Individual lobe margins are sometimes deeply cut. Their upper surface is unwrinkled and brownish-grey when dry. Concave apothecia are very numerous, ≤1.5 mm diam.; thalline margin distinct, beige, paler than the brownish disc.

L. intermedium mostly resembles diminutive specimens of *L. gelatinosum* (With.) J.R. Laundon, which, however, has larger lobes distinctly wrinkled at the upper surface and more brownish apothecial discs (Jørgensen 1994).

The material presented above differs slightly from the species description by Jørgensen (1994) and Gilbert & Jørgensen (2009) in the size of squamules and apothecia, which are larger here. Furthermore, in the Polish specimen the upper thallus surface is rather smooth, in contrast to the weakly wrinkled lobes given as a diagnostic feature for *L. intermedium*.

Specimen examined: **POLAND. Małopolska Province**, N slope of massif, Czatoża Forest, fs no. 16a [49°35′44.7″N / 19°28′57.2″E], alt. 900 m, on the base of a mossy old beech within ancient Carpathian beech forest, 29.10.2008, leg. M. Węgrzyn 4151 (KRA).

Ecology & distribution: This species is widespread in Europe (Jørgensen 1994; Gilbert & Jørgensen 2009) but has been reported only twice in Poland from the Gorce Mts in the Carpathians (Czarnota 1997) and the Góry Kaczawskie Mts in the Sudetes

(Kossowska 2008), where it was found on a clayey soil overlying calcareous sandstone and on mossy rocks in deeply shaded places (Czarnota 1997; Kossowska 2008). In the Babia Góra Massif, *L. intermedium* occurs on the base of a mossy old beech in the best conserved fragment of an ancient Carpathian beech forest in this massif. Such a substrate is rarely reported elsewhere.

Although *L. intermedium* was discovered in the Polish Carpathians no more than 20 years ago (Czarnota 1997), reports from the Gorce Mts should be regarded as historical. The 1997 flood completely destroyed all known *L. intermedium* sites in localities that region. Its new Babia Góra Massif locality may be the only site left in the Polish Carpathians.

Micarea byssacea (Th. Fr.) Czarnota, Guz.-Krzemiń. & Coppins

Specimen examined: **POLAND. Małopolska Province**, N slope of Sokolica hill, fs no. 18b [49°35′21.5″N / 19°33′39.1″E], alt. 1120 m, on decaying wood of decorticated *Picea abies* within a montane spruce-fir forest, 10.6.2009, leg. P. Czarnota 6005 (GPN).

Ecology & distribution: Recently segregated from the *Micarea micrococca* complex, this species is characterized in detail by Czarnota & Guzow-Krzemińska (2010). It is poorly known under the name *M. byssacea*, but is probably a more common epixylic or epiphytic lichenized fungus in lowland forests; in the Western Carpathians it is widespread, but uncommon (Czarnota unpubl.).

Micarea leprosula (Th. Fr.) Coppins & A. Fletcher

Specimen examined: **POLAND. Małopolska Province**, Gówniak hill, by tourist track [49°34′34″N / 19°32′39″E], alt. 1608 m, on soil, decaying bryophytes, plant debris and terricolous lichens in alpine zone, 11.11.2005, leg. P. Czarnota 4598 (GPN).

Ecology & distribution: This terricolous alpine species was reported several times from the Polish Western Beskidy Mts, but its large population is located in the neighbouring Tatra Mts (Czarnota 2007). For taxonomic notes see also under *Helocarpon crassipes*.

Micarea lithinella (Nyl.) Hedl.

Specimens examined (selected; all on sandstone shaded pebbles): **POLAND. Małopolska Province**, SE slope of Kępa hill, fs no. 25c [49°34′29.6″N / 19°33′40.4″E], alt. 1360 m, 26.8.2009, leg. P. Czarnota 6351 (GPN); SE slope of Sokolica, fs no. 25c [49°34′51.7″N / 19°34′06.5″E], alt. 1300 m, 24.5.2009, leg. M. Węgrzyn 3170 (KRA); N slope of Sokolica hill, fs no. 18c [49°35′16.0″N / 19°33′54.0″E], alt. 1200 m, 6.6.2009, leg. M. Węgrzyn 4155 (KRA); N slope Diablak Mt., W of Sulowa Cyrhla, fs no. 70c [49°35′56.7″N / 19°31′35.4″E], alt. 830 m, 5.8.2010, leg. M. Węgrzyn 4158 (KRA).

Ecology & distribution: This epilithic species is a common early colonizer of sandstone pebbles over root systems of wind-throws within montane forests, as indicated recent studies in other ranges of the Western Carpathians (Czarnota unpubl.).

Micarea nowakii Czarnota & Coppins

Specimens examined: **POLAND. Małopolska Province**, below Brona Pass, fs no. 24a [49°35′09.6″N / 19°30′49.1″E], alt. 1235 m, 14.7.2009, leg. P. Czarnota 6212 (GPN); N slope of Sokolica, fs no. 18c [49°35′15.0″N / 19°33′49.7″E], alt. 1200 m, 26.8.2009, leg. P. Czarnota 6307 (GPN); E slope of Sokolica hill, fs no. 25a [49°35′08.8″N / 19°34′05.9″E], alt. 1280 m, 9.6.2009, leg. P. Czarnota 6023 (GPN); S slope of Kępa hill, fs no. 26h [49°34′09.3″N / 19°33′03.2″E], alt. 1365 m, 17.7.2009, leg. P. Czarnota 6086 (GPN).

Ecology & distribution: This recently described species (Czarnota 2007), a member of the *Micarea prasina* group (Czarnota & Guzow-Krzemińska 2010), appears to be an early colonizer of decorticated logs of spruce wind-throws in the upper montane zone of Babia Góra Massif. It prefers well-lit places, especially forest areas destroyed by the bark beetle *Ips typographus*. The present data extend the range of *M. nowakii* to other parts of Western Carpathians, being additional to its first discoveries in the Gorce Mts and Beskid Mały Mts (Czarnota 2007).

A realistic world distribution of *M. nowakii* is as yet unknown due to its phenetic similarity to *M. denigrata*, *M. misella*, and dark coloured morphotypes of *M. prasina*. It differs from *M. denigrata* by its usually simple and shorter ascospores and lack of gyrophoric acid, from *M. misella* by its wider conidia (similar to *M. denigrata*) and the presence of micareic acid, and from *M. prasina* by its usually numerous black, barrel-like and aggregated pycnidia, and lack of thallus composed of goniocysts. *M. nowakii* is also reported from Sweden (Svensson & Westberg 2010).

Peltigera leucophlebia (Nyl.) Gyeln.

Specimen examined: **POLAND. Małopolska Province**, N slope of Diablak hill, fs no. 22m [49°34′23.2″N / 19°31′32.0″E], alt. 1620 m, on plant debris in *Salix silesiaca* community, 7.8.2009, leg. M. Węgrzyn 3198 (KRA).

Ecology & distribution: This arctic-boreal-alpine species is widespread in the northern hemisphere but is very rare in the Western Carpathians, except in the subalpine and alpine zones of the Tatra Mts (Lisická 2005). From other Polish parts of the Western Carpathians, it has only been reported on three occasions growing in lower forested mountain zones (Bielczyk 2003). In the Babia Góra Massif, *P. leucophlebia* occurs above timberline, where terricolous and mossy habitats are much more suitable for the species.

Physcia caesia (Hoffm.) Hampe ex Fürnr.

Specimen examined: **POLAND. Małopolska Province**, NW slope of Mała Babia Góra hill, Czarna Cyrhel glade [49°35′50.0″N / 19°29′8.8″E], alt. 880 m, on concrete, 23.6.2009, leg. M. Węgrzyn 4253 (KRA).

Physcia tenella (Scop.) DC.

Specimen examined: **POLAND. Małopolska Province**, N slope of Diablak Mt., Sulowa Cyrhla glade [49°36′04.9″N / 19°32′14.4″E], alt. 840 m, on bark of *Salix* sp., 3.5.2010, leg. M. Węgrzyn 4162 (KRA).

Physconia grisea (Lam.) Poelt

Specimen examined: **POLAND. Małopolska Province**, N foot of Diablak Mt., Zawoja Markowa, fs no. 72d [49°36′29.8″N / 19°31′03.5″E], alt. 740 m, on bark of roadside *Fraxinus excelsior*, 14.5.2010, leg. M. Węgrzyn 4219 (KRA).

Placynthiella oligotropha (J.R. Laundon) Coppins & P. James

Specimens examined: **POLAND. Małopolska Province**, below Brona Pass, fs no. 24a [49°35′10.8″N / 19°30′50.5″E], alt. 1230 m, 14.7.2009, leg. P. Czarnota 6173 (GPN); ibid., [49°35′10.1″N / 19°30′47.1″E], alt. 1240 m, 14.7.2009, leg. P. Czarnota 6202 (GPN); N foot of Diablak Mt., Zawoja Markowa, fs no. 77a [49°36′13.8″N / 19°30′22.5″E], alt. 815 m, 6.8.2010, leg. M. Węgrzyn 4217 (KRA).

Ecology & distribution: In the Babia Góra Massif this terricolous species prefers well-lit soil over root systems of wind-throws, similar to those in other ranges of Western

Carpathians (Czarnota unpubl.). In these habitats it is a frequent member of a pioneer lichen biota.

Porina chlorotica (Ach.) Müll. Arg.

Specimen examined: **POLAND. Małopolska Province**, N of Markowe Szczawiny shelter house, fs no. 73b [49°35′49.3″N / 19°31′13.1″E], alt. 880 m, on sandstone pebble under root system of fallen *Picea abies* within Carpathian beech forest, 2.7.2009, leg. P. Czarnota 6125 (GPN).

Porpidia soredizodes (Lamy) J.R. Laundon

Specimens examined: **POLAND. Małopolska Province**, N slope of Diablak Mt., fs no. 22a [49°34′54″N / 19°31′39.9″E], alt. 1220 m, 7.8.2009, leg. P. Czarnota 6250 (GPN); ibid., [49°34′36.6″N / 19°31′23.00″E], alt. 1370 m, 21.6.2009, leg. M. Węgrzyn 3323 (KRA); ibid., fs no. 10g [49°34′56.7″N / 19°31′54.8″E], alt. 1170 m, 3.7.2009, leg. P. Czarnota 6145 (GPN).

Ecology & distribution: This saxicolous species is frequent in the Polish Western Carpathians on various acidic rocks (Jabłońska 2010). However, since it resembles *Porpidia tuberculosa* (Sm.) Hertel & Knoph, also abundant there, correct determination of both species requires analyses of their secondary compounds. In the Babia Góra Massif, *P. soredizodes* has been found on sandstone rocks in forested zones.

Psilolechia clavulifera (Nyl.) Coppins

Specimens examined (selected): **POLAND. Małopolska Province**, N slope of Diablak Mt., fs no. 22a [49°34′46.4″N / 19°31′34.3″E], alt. 1300 m, 7.8.2009, leg. P. Czarnota 6275 (GPN); N slope of Sokolica hill, fs no. 7a [49°35′27.1″N / 19°33′28.5″E], alt. 1090 m, 21.7.2009, leg. P. Czarnota 6248 (GPN); E slope of Sokolica hill, fs no. 25a [49°35′09.5″N / 19°34′05.8″E], alt. 1280 m, 9.6.2009, leg. P. Czarnota 6029 (GPN); SE of Sokolica hill, fs no. 25c [49°34′21.4″N / 19°33′52.4″E], alt. 1210 m, 17.7.2009, leg. P. Czarnota 6216 (GPN); ibid., [49°34′51.7″N / 19°34′06.5″E], alt. 1300 m, 24.5.2009, leg. M. Węgrzyn 3183 (KRA).

Ecology & distribution: Recently Czarnota & Kukwa (2008b) presented a modern distribution of *P. clavulifera* in Poland that shows the best habitats as being roots, soil, and acidic rocks under root systems of wind-throws. The same is also observed in the Babia Góra Massif, confirming the species as an early colonizer of such habitats.

Pycnora praestabilis (Nyl.) Hafellner

In Central Europe, *P. praestabilis* is reported as a separate species resembling the Scandinavian *P. xanthococca;* however the phenetic characters are not so distinct. In the Western Carpathians, *P. praestabilis* very frequently grows in association with *P. sorophora,* which is sorediate and usually sterile but chemically similar. Some morphs of both species seem to be transient and the small conidial size differences regarded as diagnostic (Timdal 1984) are not necessarily convincing in identification. Moreover, pycnidia are not always present in *P. sorophora*; thus, separation of the species will probably rely on future molecular evidence. Here, *P. praestabilis* is recognized only as a fertile, non-sorediate *Pycnora* morphotype.

Specimens examined (selected): **POLAND. Małopolska Province**, N slope of Diablak Mt., fs no. 22a [49°34′44.5″N / 19°31′32.2″E], alt. 1335 m, 7.8.2009, leg. P. Czarnota 6256 (GPN); below Brona Pass, fs no. 24a [49°35′10.1″N / 19°30′47.2″E], alt. 1240 m, 14.7.2009, leg. P. Czarnota 6178 (GPN); S of Sokolica hill, fs no. 25c [49°34′58.2″N / 19°33′48.6″E], alt. 1285 m, 1.7.2009, leg. P. Czarnota 6110 (GPN); SE slope of Gówniak hill, fs no. 27f [49°34′4.2″N / 19°33′02.1″E], alt. 1350 m, 28.5.2009, leg. M. Węgrzyn 3362 (KRA).

Ecology & distribution: In the Babia Góra Massif, *P. praestabilis* frequently occurs on wood of decorticated *Picea abies* trunks, but mostly within well-lit upper montane spruce forest destroyed by the activity of the bark beetle *Ips typographus*.

Pycnora sorophora (Vain.) Hafellner

Specimens examined (selected): **POLAND. Małopolska Province**, N slope of Diablak Mt., fs no. 23a [49°34′44.6″N / 19°31′17.6″E], alt. 1330 m, 7.8.2009, leg. P. Czarnota 6269 (GPN); N slope of Sokolica hill, fs no. 7a [49°35′22.6″N / 19°33′07.5″E], alt. 1080 m, 25.8.2009, leg. P. Czarnota 6331 (GPN); below Brona Pass, fs no. 24a [49°35′10.1″N / 19°30′47.2″E], alt. 1240 m, 14.7.2009, leg. P. Czarnota 6179 (GPN); S of Sokolica hill, fs no. 25c [49°34′58.2″N / 19°33′48.6″E], alt. 1285 m, 1.7.2009, leg. P. Czarnota 6111 (GPN); SE slope of Kępa hill, fs no. 25g [49°34′32.9″N / 19°33′30.1″E], alt. 1440 m, 18.9.2010, leg. M. Węgrzyn 4212 (KRA).

Ecology & distribution: In the Babia Góra Massif, *P. sorophora* is more frequent than *P. praestabilis*, occupying similar woody habitats, as well as the bark of Norway spruces, but is not strictly confined to upper montane stands (see above).

Strangospora moriformis (Ach.) Stein

Specimens examined (selected): **POLAND. Małopolska Province**, E slope of Sokolica hill, fs no. 25a [49°35′02.8″N / 19°34′05.6″E], alt. 1220 m, 1.7.2009, leg. P. Czarnota 6112 (GPN); ibid., [49°35′09.6″N / 19°34′08.6″E], alt. 1265 m, 9.6.2009, leg. P. Czarnota 5989 (GPN); below Brona Pass, fs no. 24a [49°35′10.5″N / 19°30′47.4″E], alt. 1240 m, 14.7.2009, leg. P. Czarnota 6191 (GPN); N slope of Diablak Mt., fs no. 22a [49°34′48.2″N / 19°31′34.1″E], alt. 1270 m, 7.8.2009, leg. P. Czarnota 6260 (GPN); E of Markowe Szczawiny shelter house, fs no. 10d [49°35′26.0″N / 19°29′44.5″E], alt. 1105 m, 4.9.2010, leg. M. Węgrzyn 4154 (KRA).

Ecology & distribution: *Strangospora moriformis* was formerly rarely reported from Western Carpathians, including the Tatra Mts (Bielczyk 2003, Lisická 2005), but recent investigations show that this species is common in dead (destroyed by the bark beetle) parts of upper montane spruce forests throughout the Polish Western Beskidy Mts (Czarnota unpubl.). It is an early colonizer of such natural woody substrates associated, for example, with *Mycocalicium subtile*, *Lecanora subintricata,* and *Lecidea turgidula*. It is interesting that this species has not been found in similar habitats in the Polish Tatra Mts, despite recent intensive field explorations.

Trapelia corticola Coppins & P. James

Specimens examined (selected): **POLAND. Małopolska Province**, N slope of Sokolica hill, fs no. 18b [49°35′21.5″N / 19°33′39.1″E], alt. 1120 m, 10.6.2009, leg. P. Czarnota 6006 (GPN); N of Markowe Szczawiny shelter house, fs no. 74b [49°35′48″N / 19°31′12.4″E], alt. 890 m, 2.7.2009, leg. P. Czarnota 6129 (GPN); SE slope of Sokolica hill, fs no. 25c, [49°34′51.7″N / 19°34′06.5″E], alt. 1300 m, 24.5.2009, leg. M. Węgrzyn 3171 (KRA).

Ecology & distribution: A modern distribution of this sterile, sorediate species in Poland is presented by Czarnota & Kukwa (2009); based on this and recent field data, *T. corticola* seems to be common throughout the Western Carpathians (Czarnota unpubl.) but is usually found in ancient or natural forest communities with abundant standing or fallen 'dead wood'.

Trapelia obtegens (Th. Fr.) Hertel

Specimens examined: **POLAND. Małopolska Province**, S of Sokolica hill, fs no. 25c [49°35′01.9″N / 19°33′55.8″E], alt. 1260 m, 1.7.2009, leg. P. Czarnota 6089 (GPN); ibid., [49°34′51.7″N / 19°34′06.5″E], alt. 1300 m, 24.5.2009, leg. M. Węgrzyn 3178 (KRA); SE slope of Kępa hill, fs no. 25c, [49°34′48.0″N / 19°34′06.8″E], alt. 1290 m, 16.9.2009, leg. P. Czarnota 6352 (GPN).

Ecology & distribution: This epilithic early colonizer is usually found on acidic rocks in well-lit places. In forested parts of the Western Carpathians, including the Babia Góra Massif, *T. obtegens* is mostly confined to small sandstone pebbles lying on root systems of wind-throws.

Trapeliopsis glaucolepidea (Nyl.) Gotth. Schneid. Plate 1h

This species is recognized under two different morphotypes: (1) as *T. glaucolepidea* forming more or less distinct squamules with lip-shaped soralia and (2) as *T. percrenata* (Nyl.) Gotth. Schneid. producing punctiform soralia at a very early thallus developmental stage and thus never distinctly squamulose. Recent ITS analyses (Palice & Printzen 2004) support previous assumptions that both morphotypes could be conspecific (Palice 1999). In tropical populations *T. glaucolepidea* was found to be usually squamulose and often fertile but in Europe both phenetic forms are found and are almost exclusively sterile (Palice & Printzen 2004). It is interesting that European squamulose forms occur in regions with high precipitation (western Europe and mountains in the Central Europe) growing on a strongly decayed wood or humus, while the '*T. percrenata*' morphotypes occur on somewhat harder wood or are sometimes epiphytic and much more widespread in European lowlands.

The small, almost only sorediate stage of the species mostly resembles *Trapelia corticola*, another lichen found in similar habitats throughout Europe. The two morphologically similar species are composed of separate, punctiform, bright green to whitish-green soralia but differ in their chemistry. *Trapelia corticola* produces gyrophoric acid, which can be detected in the simple spot test reaction C+ red, while *Trapeliopsis glaucolepidea* soralia are C–. There are also 'soraliate' epixylic *Micarea prasina* forms that may resemble the punctiform *T. glaucolepidea* morphotypes (Czarnota unpubl.). In such cases, both species can be also identified based on their chemistry, although the micareic acid present in *M. prasina* is only detected by chromatography. The squamulose forms of *T. glaucolepidea* are very similar to the young thalli of many *Cladonia* taxa that often grow together in the same place. Here the presence of lip-shape soralia diagnoses *T. glaucolepidea*.

Specimens examined: **POLAND. Małopolska Province**, N slope of Diablak Mt., fs no. 23b [49°34′57.6″N / 19°31′13.2″E], alt. 1265 m, 7.8.2009, leg. P. Czarnota 6278 (GPN); E slope of Sokolica hill, fs no. 25a [49°35′07.9″N / 19°34′06.4″E], alt. 1290 m, 9.6.2009, leg. P. Czarnota 6012 (GPN).

Ecology & distribution: On the Polish side of the Carpathians, *T. glaucolepidea* has been reported only four times from the neighbouring Gorce Mts and Tatra Mts (Czarnota & Kukwa 2004; Czarnota 2010), but it is a widespread species, known also from the Slovak and Ukrainian Carpathians (Palice & Printzen 2004; Palice et al. 2006; Vondrák et al. 2010). In the Babia Góra Massif, it is found on humus and strongly decayed wood over root systems of up-ended spruces in natural upper montane spruce stands. Only the squamulose morphotype has been observed there and in the Tatra Mts, whereas the '*T. percrenata*' form dominates in the Gorce Mts. In the first two ranges *T. glaucolepidea* has been recorded above 1200 m a.s.l., while in Gorce Mts its localities do not exceed 1020 m.

Trapeliopsis pseudogranulosa Coppins & P. James

Specimens examined (selected): **POLAND. Małopolska Province**, N slope of Sokolica hill, fs no. 6d [49°35′27.7″N / 19°33′42.5″E], alt. 1060 m, 25.8.2009, leg. P. Czarnota 6294 (GPN); ibid., fs no. 7a [49°35′24.3″N / 19°33′12.7″E], alt. 1060 m, 21.7.2009, leg. P. Czarnota 6228 (GPN); N of Markowe Szczawiny shelter house, fs no. 73b [49°35′49.3″N / 19°31′13.1″E], alt. 880 m, 2.7.2009, leg. P. Czarnota 6127 (GPN).

Ecology & distribution: A common species throughout the Carpathians, being mostly found in the Babia Góra Massif on decaying wood of wind-throws in shady and moist forests.

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Resupinate polypores from mixed ombrophilous forests in southern Brazil

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Abstract — During a survey of polypore fungi in the municipality of São Francisco de Paula, Rio Grande do Sul State, Brazil, 24 resupinate species were identified. *Trechispora mollusca*, *Antrodia variiformis*, *Diplomitoporus venezuelicus*, and *Flaviporus subundatus*, rare species in Brazil, are fully described, discussed and illustrated. Comments are also given on *Fomitiporia maxonii*, recorded for the first time from Rio Grande do Sul State. An identification key and a checklist of the species are presented.

Key words — *Polyporaceae,* fungal diversity, neotropics

Introduction

Polypore fungi are characterized by a tubular hymenophore and being predominantly xylophilous, but they can also grow on soil or plant litter and therefore are extremely important in nutrient and energy cycling (Webster & Weber 2007). Wood-decay fungi can be divided into two large groups —white rot fungi that decompose cellulose, hemicellulose, and lignin and brown rot fungi that decompose only cellulose and hemicellulose. Wood decay fungi may cause damage when they attack wood, reducing its commercial value, and when they parasitise species of economic interest, causing a slump in productivity (Wright & Albertó 2006).

In polypores, both macro and microscopic characters are highly variable. Macroscopically, basidiomes can be resupinate or pileate, but transitions between these forms can also be observed (Ryvarden 1991). Although macro- and microscopic characters are very important in the characterization of taxa, polypores do not form a monophyletic group, and several cases of morphological convergence exist (Hibbett & Binder 2002).

Since Rick's pioneer study of macroscopic fungi, including polypores, in the state of Rio Grande do Sul (Fidalgo 1962, Rick 1960), several other

taxonomic studies have been undertaken in the State on that group (Silveira & Guerrero 1991, Groposo & Loguercio-Leite 2002, Coelho et al. 2006, Reck & Silveira 2008). Silveira et al. (2008) surveyed the pileate polypores from the São Francisco de Paula National Forest. Our survey of resupinate polypore species of São Francisco de Paula continues this study, while expanding knowledge of fungi in the region.

Materials & methods

Most examined specimens were collected from April 2009 to October 2010 in the mixed ombrophilous forest of the municipality of São Francisco de Paula, in northeastern Rio Grande do Sul State, southern Brazil. Specimens previously collected in the region were also included in this study. The mixed ombrophilous forest is characterized by subtropical vegetation with the presence of the coniferous tree *Araucaria angustifolia* (Bertol.) Kuntze. The municipality of São Francisco de Paula is located about 900m above sea level and has an area of 3274 km^2. The climate in the region is of the Cfb type, according to Köppen Classification, with rainfall high in all months (average of 2252 mm) and average annual temperature of 14.5 °C (Mota 1951, Backes 1999).

The basidiomes collected were analyzed macroscopically and later dried at room temperature. For microscopy analysis, freehand sections of the basidiomes were mounted on microscope slides with 3% KOH solution and 1% phloxine solution. Amyloid or dextrinoid reactions were observed in Melzer's reagent. Drawings of the microstructures were made with the aid of a drawing tube. All specimens were deposited at ICN herbarium (Universidade Federal do Rio Grande do Sul, Brazil).

Taxonomy

Key to species of resupinate polypores recorded in São Francisco de Paula

1a. Hyphal system monomitic 2
1b. Hyphal system di–trimitic 7

2a. Generative hyphae with clamps 3
2b. Generative hyphae simple septate 4

3a. Encrusted cystidia present in the hymenium ..... *Trechispora regularis*
3b. Cystidia absent ..... *Trechispora mollusca*

4a. Basidiomes white to cream, very fragile, basidiospores cylindrical ... *Ceriporia viridans*
4b. Basidiomes isabelline to pinkish, tough, basidiospores globose to subglobose 5

5a. Cystidia absent in the trama and hymenium, only with encrusted hyphal ends present in the dissepiments ..... *Rigidoporus crocatus*
5b. Cystidia present in the trama or hymenium 6

6a. Pore surface pale orange-pink to vivid pink, becoming smoke gray to dark brown after drying, cystidia usually present in the trama and hymenium, basidiospores subglobose ..... *Rigidoporus vinctus*
6b. Pore surface cream to isabelline, becoming only slightly darker after drying, cystidia restricted to the trama, basidiospores globose ..... *Rigidoporus undatus*

7a. Generative hyphae with simple septa 8
7b. Generative hyphae with clamps 12

8a. Basidiomes pinkish, not becoming black in KOH, basidiospores very small (2.5–3 × 2–2.5 µm) ..... *Flaviporus subundatus*
8b. Basidiomes in shades of brown, becoming black in KOH, basidiospores larger 9

9a. Basidiospores non-dextrinoid, ellipsoid, rusty brown ..... *Fulvifomes umbrinellus*
9b. Basidiospores dextrinoid, globose, hyaline to pale yellow 10

10a. Setae lacking ..... *Fomitiporia maxonii*
10b. Setae present 11

11a. Pores 7–10/mm, basidiospores 3.5–5.0 µm in diam., setae 11–27 µm long ..... *Phellinus bambusarum*
11b. Pores 2–5/mm, basidiospores 4.5–7 µm in diam., setae 12–40 µm long ..... *Fomitiporia sanctichampagnatii*

12a. Basidiospores ornamented, 10–15 µm long ..... *Pachykytospora papyracea*
12b. Basidiospores smooth, less than 10 µm long 13

13a. Basidiomes perennial, basidiospores dextrinoid, truncate and thick-walled ..... *Perenniporia medulla-panis*
13b. Basidiomes annual, basidiospores non-dextrinoid, different shaped and thin-walled 14

14a. Cystidia present 15
14b. Cystidia absent 17

15a. Pore surface yellowish becoming red when bruised, cystidia finely encrusted ..... *Junghuhnia carneola*
15b. Pore surface differently colored, cystidia coarsely encrusted 16

16a. Pore surface cinnamon to brick-red, basidiospores narrowly ellipsoid to subcylindrical ..... *Junghuhnia meridionalis*
16b. Pore surface cream to pale orange-pink, basidiospores broadly ellipsoid to ovoid ..... *Junghuhnia nitida*

17a. Pore surface lilac grey, vegetative hyphae dextrinoid, basidiomes reddening the substrate ..... *Tinctoporellus epimiltinus*
17b. Pore surface white to beige, vegetative hyphae nondextrinoid, basidiomes not reddening the substrate 18

18a. Vegetative hyphae weakly amyloid and dissolving in KOH *Cinereomyces lindbladii*
18b. Vegetative hyphae negative in Melzer's reagent and not dissolving in KOH 19

19a. Scattered hyphae with an apical cap of crystals, spores 0.5–1 µm wide, lunate ..... *Sidera lenis*
19b. No hyphae with encrusted cap, spores wider, ellipsoid to cylindrical 20

20a. Pores 6–9/mm, basidiospores 1–2 µm wide ..... *Diplomitoporus venezuelicus*
20b. Pores 1–5/mm, basidiospores more than 2 µm wide 21

21a. Causing a white rot, basidiospores ellipsoid, capitate cystidioles present in the hymenium 22
21b. Causing a brown rot, basidiospores cylindrical, capitate cystidioles absent 23
22a. Pores mostly irregular, 1–3/mm, basidiospores 5–6.5 × 3–4 µm ..... *Schizopora paradoxa*
22b. Pores regular, 3–5/mm, basidiospores 3.5–5 × 2.5–3.5 µm ... *Schizopora flavipora*
23a. Pores mostly round to angular, sometimes slightly split and elongated, basidiospores 8–12 × 3.5–5 µm ..... *Antrodia albida*
23b. Pore surface mostly irpicoid to daedaleoid, pores strongly split, basidiospores 6.5–10 × 2–4 µm ..... *Antrodia variiformis*

Antrodia albida (Fr.) Donk, Persoonia 4(3): 339, 1966

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 18.V.2009, leg. M.C. Westphalen 183/09 (ICN 154265); 25.IX.2009, leg. M.C. Westphalen 258/09 (ICN 154353); CPCN Pró-Mata, 29.V.2009, leg. M.C. Westphalen 204/09 (ICN 154266), leg. M.C. Westphalen 213/09 (ICN 154267), leg. M.C. Westphalen 214/09 (ICN 154268).

Antrodia variiformis (Peck) Donk, Persoonia 4(3): 340, 1966 Figs 1–3, Plate 1

Basidiomes annual, resupinate, adnate, effused, separable, ≤1.5 mm thick, corky and brittle; margin sharp, cream colored, narrow, ≤1 mm wide; pore surface at first beige, drying cork colored, pale cinnamon or light brown, pores irregular, distinctly split and sinuous, irpicoid to daedaloid, 1–3 per mm; tubes cream to pale brown, ≤1 mm deep; subiculum cream to beige, often forming a darker cinnamon to brownish zone in the regions where in contact with the substrate, ≤0.5 mm thick.

Hyphal system dimitic; generative hyphae with clamps, thin-walled, hyaline, 2–4 µm wide; skeletal hyphae dominant, thick-walled to almost solid, hyaline to yellowish, straight, unbranched to dichotomously branched, 3–6 µm wide, IKI-; cystidia absent, scattered fusoid cystidioles present. Basidia clavate,

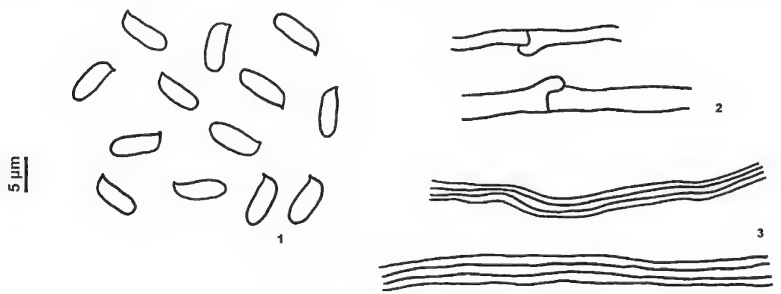

Fig. 1–3. *Antrodia variiformis* .
1. Basidiospores. 2. Generative hyphae. 3. Skeletal hyphae.

Plate 1. Basidiome of *Antrodia variiformis*. Scale bar = 1 cm

4-sterigmate. Basidiospores cylindrical, hyaline, smooth, thin-walled, (6-)6.5–10 × (2-)2.5–4 µm.

Substrata: Fallen logs of an undetermined angiosperm.

Distribution: Previously recorded from North America, Europe and Africa. This is the first record of the species from South America.

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, Hotel Veraneio Hampel, 26.V.2008, leg. M.A. Reck s.n. (ICN 154706). **CANADÁ. Quebec**, Gaspé county, Kelly's Camp, VIII.1936, leg. C.G. Riley s.n. (S F6828)

Remarks: *Antrodia variiformis* is characterized by the irregular irpicoid to daedaleoid hymenophore with large pores and the beige to cork-colored pore surface. *Antrodia albida* is a similar species differentiated by the white to cream basidiomes when fresh with more regular pores. The specimen of *A. variiformis* collected in Rio Grande do Sul State has a fully resupinate basidiome, but the species can be effused-reflexed or, more rarely, sessile (Gilbertson & Ryvarden 1986). The specimen examined from S herbarium is very similar to ours, but it has lifted margins forming, in some regions, small brown pilei. The spore size, mainly the length, of the Brazilian collection also differs from the descriptions given by other authors. Gilbertson & Ryvarden (1986) described the basidiospores of *A. variiformis* as 8–12 × 3–4.5 µm, Ryvarden & Johansen (1980) measured them 8–10(–11) × 2.5–4 µm, while according to Niemelä

(1985) they are 8–9 × 2.8–3 µm. In the S herbarium specimen the basidiospores were also slightly shorter, 7–8.5 × 2.5–3.5 µm. We believe that this variation in the spore size is normal within the species, especially when comparing collections from different regions of the world. As all the other characteristics of the collected specimen are the same as those of *A. variiformis*, we identified it as such.

Ceriporia viridans (Berk. & Broome) Donk, Meddn Bot. Mus. Herb. Rijhs Universit. Utrecht. 9: 171, 1933

Specimen examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, CPCN Pró-Mata, leg. M.C. Westphalen 345/11, 07.V.2011 (ICN 170625).

Cinereomyces lindbladii (Berk.) Jülich, Biblioth. Mycol. 85: 400, 1982

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 18.V.2009, leg. M.A. Reck 073/09 (ICN 154359); 22.VI.2009, leg. M.C. Westphalen 244/09 (ICN 154275); 25.IX.2009, leg. M.C. Westphalen 254/09 (ICN 154360), leg. M.C. Westphalen 257/09 (ICN 154361).

Diplomitoporus venezuelicus Ryvarden & Iturr., Mycol. 95(6): 1069, 2003 Figs 4–7

Basidiomes annual, resupinate, adnate, forming more or less confluent patches on the substrate, ≤1.5 mm thick, soft and somewhat watery when fresh becoming rigid upon drying; marginal sterile zone white to cream, very narrow,

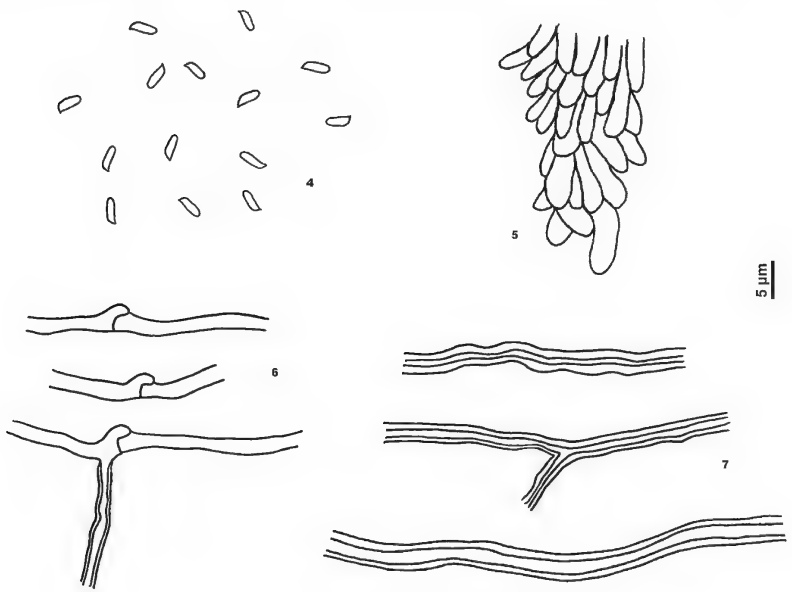

Fig. 4–7. *Diplomitoporus venezuelicus*.
4. Basidiospores. 5. Hyphal pegs. 6. Generative hyphae. 7. Skeletal hyphae.

≤0.15 mm wide; pore surface white to cream drying beige to straw-colored, pores regular, round to angular, 6–9 per mm, tubes concolorous with the pore surface, ≤1.5 mm deep; subiculum white to cream, very thin, ≤0.35 mm thick.

Hyphal system dimitic; generative hyphae with clamps, hyaline and thin-walled, 2–3 µm in diam.; skeletal hyphae dominating, hyaline and thick-walled, mostly unbranched, 2–4 µm in diam.; hyphal pegs scattered in the hymenium, difficult to observe. Basidia clavate, 4-sterigmate; basidiospores cylindrical, hyaline, thin-walled, smooth, (3.5–)4–4.5 ×1–1.5(–2) µm.

Substrata: Fallen logs of an undetermined angiosperm.

Distribution: Known from Venezuela, Colombia and Brazil.

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, CPCN Pró-Mata, leg. M.C. Westphalen 347/11, 07.V.2011 (ICN 170624). **COLOMBIA. Dpt. Magdalenha**, Sierra Nevada de Santa Marta, Reserva Forestal San Lorenzo, leg. L. Ryvarden 16195, 17-19.VI.1978 (O 918147).

Remarks: *Diplomitoporus venezuelicus* is characterized by the white to cream resupinate basidiomes with small pores and the small cylindrical basidiospores. The specimen examined has a few hyphal pegs, although these are sometimes difficult to observe. This is the first report of hyphal pegs for this species.

Flaviporus subundatus (Murrill) Ginns, Canad. J. Bot. 58(14): 1587, 1980 Figs 8–10

Basidiomes annual to perennial, resupinate, adnate, ≤3 mm thick, tough when fresh, very hard and resinous when dried, usually cracking into pieces; margin very narrow to absent; pore surface flesh-colored to pinkish, pores regular, circular to angular, 8–10 per mm; tubes concolorous with the pore surface, ≤2.5 mm thick; subiculum very thin to absent, ochraceous to brownish, ≤0.5 mm thick.

Hyphal system dimitic, hyphae strongly agglutinated; generative hyphae with simple septa, hyaline, thin to thick-walled, 2.5–5 µm in diam.; skeletal hyphae dominating, hyaline, thick-walled, unbranched, ≤5.5 µm thick.

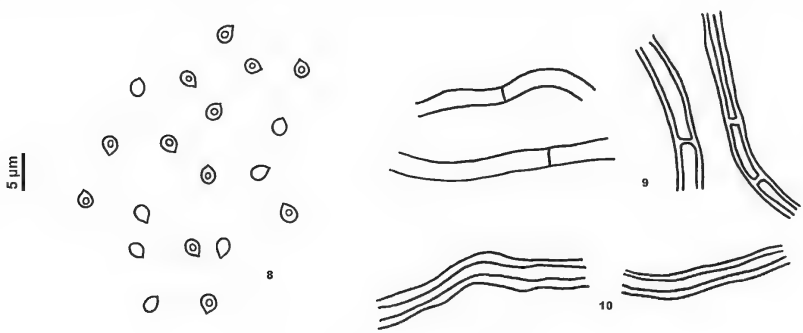

Fig. 8–10. *Flaviporus subundatus*.
8. Basidiospores. 9. Generative hyphae. 10. Skeletal hyphae

Cystidia present, embedded in the trama and very difficult to separate, usually seen only as large club-like crystalline structures. Basidia clavate, 4-sterigmate; basidiospores very small, subglobose to broadly ellipsoid, hyaline, thin-walled, smooth, 2.5–3 × 2–2.5 µm.

Substrata: Fallen logs of an undetermined angiosperm.

Distribution: Pantropical.

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, Hotel Veraneio Hampel, leg. M.C. Westphalen 312/10, 27.III.2010 (ICN 154701). **CUBA. Guantánamo**, Baracoa, El Yunque Mountain, leg. L.M. Underwood & F.S. Earle 1168, III/1903 (BPI US0243504, isotype of *Poria subundata*).

Remarks: *Flaviporus subundatus* can be easily identified by the resinous-hard resupinate basidiomes, pinkish color, very small pores and basidiospores, generative hyphae with simple-septa, and incrusted cystidia embedded in the trama. Ryvarden (2007) recently transferred this species from *Flaviporus* Murrill to *Junghuhnia* Corda. However, both genera (sensu typorum) are characterized by the presence of clamp-connections in the generative hyphae. The basidiome color and absence of clamp-connections in *F. subundatus* are features that come close to those of *Rigidoporus*, but the latter usually has a monomitic hyphal system and larger basidiospores. We consider that neither *Flaviporus* nor *Junghuhnia* are appropriate to accommodate this species and that further study involving molecular data is necessary to resolve its generic placement.

Fomitiporia maxonii Murrill, N. Amer. Fl. 9(1): 11, 1907

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 24.IV.2009, leg. M.C. Westphalen 161/09 (ICN 154308); CPCN Pró-Mata, 26.IX.2009, leg. M.C. Westphalen 270/09 (ICN 154374), leg. M.C. Westphalen 272/09 (ICN 154375).

Remarks: *Fomitiporia maxonii* is very similar to *F. punctata* (P. Karst.) Murrill [≡ *Phellinus punctatus* (P. Karst.) Pilát], differing only by basidiospore size (*F. maxonii*, 5–6.5 × 4.5–6 µm; *F. punctata*, 6.5–8.5 × 5.5–7 µm). Ryvarden (1985) considered them to be synonymous. However, more recently, phylogenetic studies by Decock et al. (2007) show that they represent two different taxa and that *F. maxonii* is widely distributed in the neotropics, including Brazil. Even though this is the first confirmed record of the species from Rio Grande do Sul, it is probable that many collections from the State identified as *Phellinus punctatus* actually represent *F. maxonii*.

Fomitiporia sanctichampagnatii G. Coelho, R.M. Silveira & Rajchenb., Fung. Diversity 36: 3, 2009

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, CPCN Pró-Mata, 06.VI.2006, leg. G. Coelho 493-1 (ICN 139201), leg. G. Coelho 493-2 (ICN 139202), leg. G. Coelho 493-3 (ICN 139203).

Fulvifomes umbrinellus (Bres.) Y.C. Dai, Fung. Diversity 45: 203, 2010

Specimen examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 26-III-2010, leg. M.C. Westphalen 305/10 (ICN 154665); CPCN Pró-Mata, 26.IX.2009, leg. M.C. Westphalen 266/09 (ICN 154373).

Junghuhnia carneola (Bres.) Rajchenb., Revista Invest. Agropec. 19: 45, 1984

Specimen examined: **BRAZIL. Rio Grande do Sul**: São Francisco de Paula, CPCN Pró-Mata, 14.XI.2009, leg. M.C. Westphalen 289/09 (ICN 154468).

Junghuhnia meridionalis (Rajchenb.) Rajchenb., Austral. Syst. Bot. 16(4): 477, 2003

Specimen examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 22.VI.2009, leg. M.C. Westphalen 238/09 (ICN 154290); 26-III-2010, leg. M.C. Westphalen 295/10, (ICN 154659); leg. M.C. Westphalen 303/10 (ICN 154660).

Junghuhnia nitida (Pers.) Ryvarden, Persoonia 7(1): 18, 1972

Specimen examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, CPCN Pró-Mata, 30.V.2009, leg. M.C. Westphalen 222/09 (ICN 154296); FLONA, 22.VI.2009, leg. M.C. Westphalen 236/09 (ICN 154294), leg. M.C. Westphalen 241/09 (ICN 154295).

Pachykytospora papyracea (Cooke) Ryvarden, Norweg. J. Bot. 19: 233, 1972

Specimen examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, CPCN Pró-Mata, 29.V.2009, leg. M.C. Westphalen 219/09 (ICN 154302); 26.IX.2009, leg. M.C. Westphalen 265/09 (ICN 154369); 26-III-2010, leg. M.C. Westphalen 302/10 (ICN 154663).

Perenniporia medulla-panis (Jacq.) Donk, Persoonia 5(1): 76, 1967

Specimen examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, CPCN Pró-Mata, 30.V.2009, leg. M.C. Westphalen 224/09 (ICN 154303).

Phellinus bambusarum (Rick) M.J. Larsen, Synopsis Fung. 3: 40, 1990

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 24.IV.2009, leg. M.C. Westphalen 179/09 (ICN 154305); CPCN Pró-Mata, 29.V.2009, leg. M.C. Westphalen 217/09 (ICN 154306); 26.IX.2009, leg. M.C. Westphalen 264/09 (ICN 154372).

Rigidoporus crocatus (Pat.) Ryvarden, Occas. Pap. Farlow Herb. Cryptog. Bot. 18: 13, 1983

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, CPCN Pró-Mata, 29.V.2009, leg. M.C. Westphalen 218/09 (ICN 154320); 07.X.2010, leg. M.C. Westphalen 338/10 (ICN 154703); FLONA, 22.VI.2009, leg. M.C. Westphalen 231/09 (ICN 154316), leg. M.C. Westphalen 248/09 (ICN 154317), leg. M.C. Westphalen 253/09 (ICN 154319).

Rigidoporus undatus (Pers.) Donk, Persoonia 5(1): 115, 1967

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 24.IV.2009, leg. M.C. Westphalen 148/09 (ICN 154297); CPCN Pró-Mata, 26.VI.2010, leg. M.C. Westphalen 335/10 (ICN 154695).

Rigidoporus vinctus (Berk.) Ryvarden, Norweg. J. Bot. 19: 143, 1972

Specimen examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 24.IV.2009, leg. M.C. Westphalen 153/09 (ICN 154315).

Schizopora flavipora (Berk. & M.A. Curtis) Ryvarden, Mycotaxon 23: 186, 1985

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 24.IV.2009, leg. M.C. Westphalen 151/09 (ICN 154280); 18.V.2009, leg. M.C. Westphalen 187/09 (ICN 154281); 25.IX.2009, leg. M.C. Westphalen 259/09 (ICN 154380); CPCN Pró-Mata, 26.IX.2009, leg. M.C. Westphalen 269/09 (ICN 154381); 26.VI.2010, leg. M.C. Westphalen 337/10 (ICN 154691).

Schizopora paradoxa (Schrad.) Donk, Persoonia 5(1): 104, 1967

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 18.V.2009, leg. M.C. Westphalen 184/09 (ICN 154282); CPCN Pró-Mata, 29.V.2009, leg. M.C. Westphalen 208/09 (ICN 154283); 30.V.2009 leg. M.C. Westphalen 228/09 (ICN 154284); 26.IX.2009, leg. M.C. Westphalen 274/09 (ICN 154382).

Sidera lenis (P. Karst.) Miettinen, Mycol. Progr. 10(2): 136, 2011

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 18.V.2009, leg. M.C. Westphalen 182/09 (ICN 154321), leg. M.C. Westphalen 188/09 (ICN 154322); 22.VI.2009, leg. M.C. Westphalen 234/09 (ICN 154323), leg. M.C. Westphalen 245/09 (ICN 154324); CPCN Pró-Mata, 29.V.2009, leg. M.C. Westphalen 220/09 (ICN 154325); 26.IX.2009, leg. M.C. Westphalen 268/09 (ICN 154383).

Tinctoporellus epimiltinus (Berk. & Broome) Ryvarden, Trans. Brit. Mycol. Soc. 73: 19, 1979

Specimen examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, Hotel Veraneio Hampel, 27.III.2010, leg. M.C. Westphalen 317/10 (ICN 154671).

Trechispora mollusca (Pers.) Liberta, Canad. J. Bot. 51(10): 1878, 1974 Figs 11–12

Basidiomes annual, resupinate, adnate, ≤1 mm thick, very soft and fragile, separable from the substrata; marginal sterile zone white, cottony, arachnoid or fimbriate, sometimes slightly rhizomorphic, narrow to wide; pore surface white when fresh, drying cream, pores irregular, circular to angular, sinuous, split in some parts, 2–6 per mm; tubes concolorous with the pore surface, fragile, ≤1 mm thick; subiculum white to light cream, very thin, soft and fibrous, continuous with the trama.

Hyphal system monomitic; generative hyphae with clamps, thin-walled, branched, often ampullate at the septa, sometimes incrusted with small granules, 2–6 μm in diam., IKI-; cystidia and other sterile elements absent. Basidia clavate to cylindrical, 4-sterigmate; basidiospores ovoid to ellipsoid, hyaline, thin-walled, echinulate, 3.5–4.5 × 2.5–3.5 μm.

Substrata: Fallen logs of an unknown angiosperm.

Distribution: Circumglobal.

Specimens examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, CPCN Pró-Mata, 26.IX.2009, leg. M.C. Westphalen 276/09 (ICN 154386), leg. M.C. Westphalen 277/09 (ICN 154387).

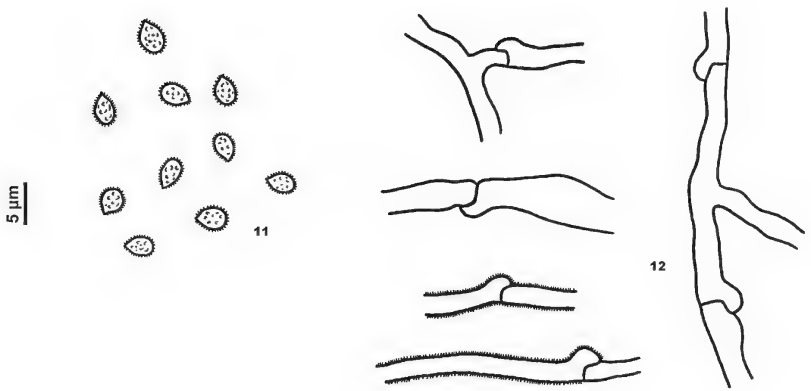

Fig. 11–12. *Trechispora mollusca*.
11. Basidiospores. 12. Generative hyphae.

Remarks: *Trechispora mollusca* is characterized by very fragile basidiomes with cottony margins and sinuous pores. Microscopically it has a monomitic hyphal system and ornamented spores. It is very similar to *T. regularis*, differing only in the absence of cystidia in the hymenium. *Trechispora mollusca* was recently recorded as new to Brazil by Drechsler-Santos et al. (2008), but their Brazilian specimen deposited in FLOR herbarium has effused-reflexed basidiomes and a flexible consistency. After microscopic examination, we identified it as *Junghuhnia undigera* (Berk. & M.A. Curtis) Ryvarden. Therefore, we consider the ICN material cited above as the first record of *T. mollusca* from Rio Grande do Sul State and Brazil.

Trechispora regularis (Murrill) Liberta, Canad. J. Bot. 51(10): 1878, 1974

Specimen examined: **BRAZIL. Rio Grande do Sul**, São Francisco de Paula, FLONA, 22.VI.2009, leg. M.C. Westphalen 246/09 (ICN 154329).

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Coltricia australica sp. nov. (*Hymenochaetales, Basidiomycota*) from Australia

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Abstract — *Coltricia australica* sp. nov. is described and illustrated from Tasmania, Australia. It is characterized by its annual and centrally stipitate basidiocarps with concentrically zonate and glabrous pilei when dry, angular pores of 3–4 per mm, and ellipsoid, thin- to thick-walled, smooth, pale yellowish, and cyanophilous basidiospores. This species is terrestrial in angiosperm forests.

Key words — *Hymenochaetaceae,* polypore, taxonomy

Introduction

Coltricia Gray, typified by *C. connata* Gray [= *C. perennis* (L.) Murrill], is a cosmopolitan genus of *Hymenochaetales* and has been well studied in Africa (Ryvarden & Johansen 1980), Asia (Núñez & Ryvarden 2000, Dai & Cui 2005, Dai et al. 2010, Dai 2010, 2012, Dai & Li 2012, Baltazar & Silveira 2012), Europe (Ryvarden & Gilbertson 1993), Neotropics (Ryvarden 2004, Baltazar et al. 2010), and North America (Gilbertson & Ryvarden 1986). *Coltricia* differs from other genera in *Hymenochaetales* by the combination of annual stipitate and fragile (when dry) basidiocarps, a monomitic hyphal system, and colored slightly to distinctly thick-walled smooth basidiospores (Dai 2010).

Coltriciella Murrill, the most morphologically similar genus to *Coltricia,* differs by its ornamented basidiospores (Dai 2010). Recent molecular data confirmed that the two genera are closely related in a strongly supported clade, but the monophyly of both genera remains unresolved (Wagner & Fischer 2002, Larsson et al. 2006).

Most species of *Coltricia* and *Coltriciella* are terrestrial or associate with strongly decayed wood. Despite the woody fruitbody texture and phylogenetic

placement within the *Hymenochaetales* comprising mostly white-rot agents of wood, several species of the two genera have been reported to be ectomycorrhizal (EcM) symbionts of different trees based on both morphological and molecular evidence (Thoen & Ba 1989, Tedersoo et al. 2007, 2010).

The stipitate and terrestrial species of *Phylloporia* Murrill, in particular *P. minutispora* Ipulet & Ryvarden, *P. spathulata* (Hook.) Ryvarden, and *P. veraecrucis* (Berk. ex Sacc.) Ryvarden, are frequently misidentified as *Coltricia* and *Coltriciella* in the field, but *Phylloporia* spp. have smaller basidiospores (<4.5 µm long and <3.5 µm wide, Zhou & Dai 2012). However, a reliable delimitation between *Phylloporia* and *Coltricia* is still unknown; for example, *Coltricia stuckertiana* (Speg.) Rajchenb. & J.E. Wright with typical *Coltricia* characters clusters within the *Phylloporia* clade (Valenzuela et al. 2011).

During a field trip in Australia, a specimen of *Coltricia* was collected and originally labeled as "*C.* cf. *cinnamomea*". Molecular evidence (Tedersoo et al. 2007) and careful microscopic examination revealed it to differ from described species and to represent a new *Coltricia* species. Description and drawings of the microscopic structures of this new species are provided.

Materials & methods

The studied specimen was deposited at the herbaria of University of Tartu (TU) and Beijing Forestry University (BJFC). The microscopic procedure follows Dai (2010). A Nikon Eclipse 80i microscope at magnification up to ×1000 was used to study the sections prepared in IKI (Melzer's reagent), KOH (5% potassium hydroxide) and CB (Cotton Blue) solutions. When presenting the variation in the size of the spores, 5% of measurements were excluded from each end of the range and are given in parentheses. The following abbreviations are used: IKI– = negative in Melzer's reagent, CB+ = cyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens and n = number of spores measured from the given number of specimens. Line drawings were made with the aid of a light tube. Special color terms follow Petersen (1996).

Taxonomy

Coltricia australica L.W. Zhou, Tedersoo & Y.C. Dai, **sp. nov.** FIGURE 1

MYCOBANK MB 800364

Differs from *Coltricia cinnamomea* in its slightly smaller basidiospores and narrower contextual hyphae.

TYPE: Australia. Tasmania, Southwest Conservation Area, Warra Long-Term Ecological Research site, ground in mixed forest of *Eucalyptus* and *Nothofagus*, 8.VIII.2006 (**holotype**, TU 103694; **isotype**, BJFC; European Nucleotide Archive accession number, AM412243 for nLSU).

ETYMOLOGY: *australica* (Lat.): refers to the country of Australia.

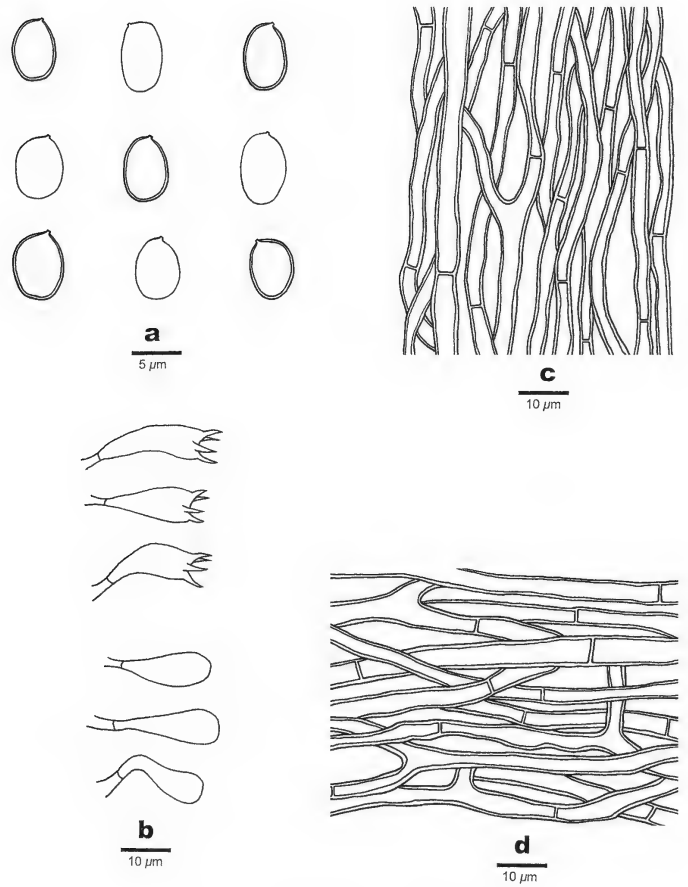

FIGURE 1. *Coltricia australica* (holotype).
a: Basidiospores. b: Basidia and basidioles. c: Hyphae from trama. d: Hyphae from context.

BASIDIOCARPS annual, centrally stipitate, gregarious. PILEUS more or less circular, infundibuliform, up to 3 cm in diam., 3 mm thick at centre. PILEAL SURFACE cinnamon to greyish brown, concentrically zonate, when dry with radial wrinkled ridges, glabrous; margin thin, acute, curving down when dry. PORE SURFACE reddish brown to greyish brown; pores angular, 3–4 per mm; dissepiments thin, entire. CONTEXT dark brown, coriaceous, up to 1 mm thick. TUBES yellowish brown, paler than the context, fragile when dry, up to 2 mm long. STIPE yellowish brown, matted, up to 3 cm long and 2 mm in diam.

HYPHAL SYSTEM monomitic; GENERATIVE HYPHAE simple septate; tissue becoming reddish brown in KOH but otherwise unchanged. CONTEXTUAL

HYPHAE pale yellowish to golden brown, thick-walled with a wide lumen, regularly branched and septate, straight, regularly arranged, 3.5–6.5 µm in diam.; hyphae in the stipe similar to those in context. TRAMAL HYPHAE pale yellowish to golden brown, thick-walled with a wide lumen, occasionally branched, regularly septate, straight, subparallel along tubes, 2.9–5.5 µm in diam. BASIDIA clavate, with four sterigmata and a simple septum at the base, 14–21 × 6–7.8 µm; basidioles in shape similar to basidia, but slightly smaller. BASIDIOSPORES broadly ellipsoid to ellipsoid, pale yellowish, thin- to thick-walled, smooth, IKI–, CB+, (5.5–)6–7.3(–7.4) × (4–)4.1–5.2(–5.3) µm, L = 6.54 µm, W = 4.77 µm, Q = 1.37 (n = 30/1).

REMARKS: The holotype specimen of *C. australica* TU103694 was subjected to sequence analysis of both nuclear large subunit ribosomal DNA (nLSU) and internal transcribed spacer (ITS) regions as described in Tedersoo et al. (2007). The ITS fragment was probably too long to yield a PCR product, but a 1009-nucleotide nLSU sequence was easily obtained. nLSU-based phylograms suggested that this specimen is closely related to a *Coltricia* specimen [MEL (Melbourne Royal Botanical Gardens Herbarium) 2059672, AM412244] also from Australia (Tedersoo et al. 2007) but unavailable for examination. Other *Coltricia* and *Coltriciella* species were phylogenetically well segregated from the two Australian specimens.

Coltricia australica macroscopically resembles *C. cinnamomea* (Jacq.) Murrill, *C. perennis, C. pyrophila* (Wakef.) Ryvarden, *C. subperennis* Y.C. Dai [nom. illegit.], and *C. weii* Y.C. Dai by sharing a central stipe, similar pores (3–4 per mm), and ellipsoid basidiospores (Dai 2010).

Coltricia cinnamomea is distinguished by slightly larger basidiospores (TABLE 1) and wider contextual hyphae (6–10 µm in diam., Dai 2010).

Coltricia perennis has longer basidiospores (TABLE 1) and wider contextual hyphae (6–9 µm in diam., Dai 2010) than *C. australica*. In the field, it is easy to differentiate the two species: *C. perennis* grows on gymnosperm forest floors (Gilbertson & Ryvarden 1986, Ryvarden & Gilbertson 1993, Baltazar et al. 2010, Dai 2010), while *C. australica* inhabits angiosperm forests.

Coltricia pyrophila differs from *C. australica* in its smaller basidiospores (TABLE 1) and loosely interwoven tramal hyphae (Dai 2010).

Coltricia subperennis is distinguished from *C. australica* by its larger basidiospores (TABLE 1) and narrower tramal hyphae (3–4 µm in diam., Dai 2010).

When dry, *Coltricia weii* has dark reddish to umber pileal surface and umber pore surface (Dai 2010), which are paler than those in *C. australica*. In addition, the basidiospores are weakly cyanophilous in *C. weii* (Dai 2010) but strongly cyanophilous in *C. australica*.

Table 1. Comparison of basidiospore sizes of six similar *Coltricia* species based on Dai (2010) and specimen examined in the present study.

Species	Basidiospores [L × W]
C. australica	6.54 × 4.77 µm, Q = 1.37
C. cinnamomea	7.18 × 5.25 µm, Q = 1.34–1.39
C. perennis	7.41 × 4.70 µm, Q = 1.49–1.67
C. pyrophila	5.20 × 3.82 µm, Q = 1.36
C. subperennis	8.25 × 5.70 µm, Q = 1.42–1.47
C. weii	6.36 × 4.98 µm, Q = 1.22–1.35

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Four new rust taxa on *Asteraceae* from Central Alborz, northern Iran

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Abstract — During the study of the rust mycobiota of Central Alborz, four new taxa (two species and two varieties) were found: *Puccinia artemisiae-chamaemelifoliae* on *Artemisia chamaemelifolia*, *P. crepidis-asadbarensis* on *Crepis asadbarensis*, *P. jaceae* var. *elbursensis* on *Centaurea behen*, and *P. punctiformis* var. *karajensis* on *Cirsium arvense*.

Key Words — *Fungi*, *Pucciniales*, taxonomy, mycology

Introduction

The Central Alborz, located in the middle Alborz chain, is situated at 35°00′–36°30′N 50°20′–53°10′E and contains several peaks above 3000 m (Gitasshenasi 2004). Due to its varied topography and climate, this area has a remarkable diversity of vegetation (Klein 1994). The rust mycobiota of Central Alborz has not yet been intensively studied. The *Asteraceae* harbor a wide range of rust fungi, especially species of the genus *Puccinia*. In Iran, so far 66 *Puccinia* spp. have been reported on hosts of 44 asteraceous genera. During the course of studies on the rust mycobiota of Central Alborz, several *Puccinia* spp. on asteraceous plants have been identified. Four of them proved to be new and are described in this paper.

Materials & methods

Several rust-infected plant specimens were collected from different parts of the Central Alborz region in northern Iran. All specimens were studied macroscopically and microscopically. Color, shape and structure of sori of all infected specimens were carefully examined by an Olympus stereomicroscope. Glycerine-Lactic acid was used in preparing microscopic slides. All preparations were studied by an Olympus BH2 Nomarski Differential Interference Contrast Microscope (DIC) at magnifications of × 400 and × 1000. 50 spores per specimen were randomly selected and measured. All

specimens are deposited in the Fungal Reference Collection of the Ministry of Jihad-e Agriculture (IRAN) located at Iranian Research Institute of Plant Protection, Tehran.

Taxonomy

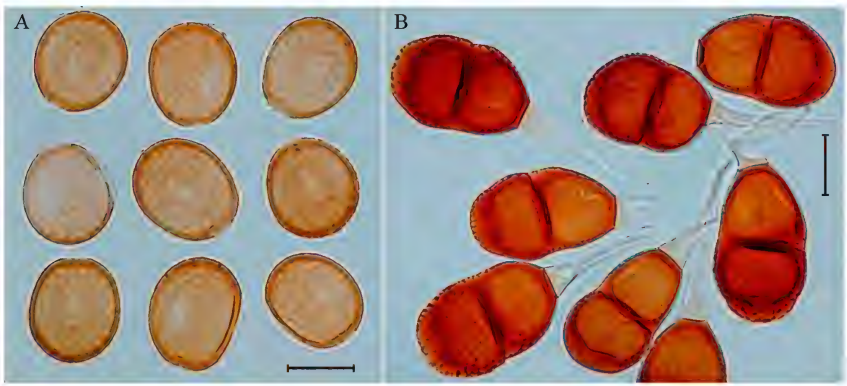

Plate 1. ***Puccinia artemisiae-chamaemelifoliae*** on *Artemisia chamaemelifolia:* A. urediniospores; B. teliospores. Scale bars = 20 µm.

Puccinia artemisiae-chamaemelifoliae Aliabadi & M. Abbasi, **sp. nov.** Plate 1

Mycobank MB 564231

Differs from *Puccinia absinthii* s. lat. by its smaller teliospores with a smaller length/width ratio.

Type: On *Artemisia chamaemelifolia* Vill.: Iran, Tehran Prov., Central Alborz, Haraz road, Hareh village, 3 km after village, alt. 2000–2200 m, II+III, 5 August 1993, leg. J.Fatehi, M.Abbasi & Z.Ghanbari (**holotype**, IRAN 14660 F).

Etymology: referring to the host species.

Spermogonia and aecia not observed. Uredinia epiphyllous and caulicolous, yellowish brown, round to oval–oblong, semi-compact; urediniospores 27.5–32.5(–37.5) × (20–)22.5–25(–27.5) µm, globoid to ovoid, wall yellowish brown, 1.5–2.5 µm thick, echinulate, with 3(–4) equatorial germ pores covered with hyaline papillae. Telia mostly epiphyllous and caulicolous, blackish brown, round to oblong, semi–compact; teliospores (37.5–)40–47.5(–50) × (22.5–)25–30 µm, ellipsoid, broadly obovoid, clavate or oblong, wall 2.5–5 µm thick at the sides and sometimes up to 7.5 µm apically, chestnut–brown, finely and densely verrucose, verrucae more visible on the upper cell of the spore and becoming smaller towards the hilum, germ pore of the upper cell approximately apical and of the lower cell below the septum, pedicel hyaline, persistent, up to 90 µm long.

Comments — Based on Braun's revision (Braun 1981), the rust on *A. chamaemelifolia* is close to *P. absinthii* s. lat. but differs from it in having smaller

teliospores and a smaller teliospore length/width ratio. *Puccinia artemisiae-chamaemelifoliae* teliospores have a mean length of 43.5 µm and a mean width of 27.5 µm, giving a mean length/width ratio of 1.6. However the mean teliospore length for *P. absinthii* is greater than 45 µm and its teliospore L/W ratio is 1.7–2.1 (Braun 1981). The only *Puccinia* species reported on *A. chamaemelifolia* is *P. chamaemelifoliae* published by Viennot-Bourgin (1958); according to the original description, only the uredinial state was present, with urediniospores 21–29 × 19–23 µm, i.e., smaller than those of *P. artemisiae-chamaemelifoliae*.

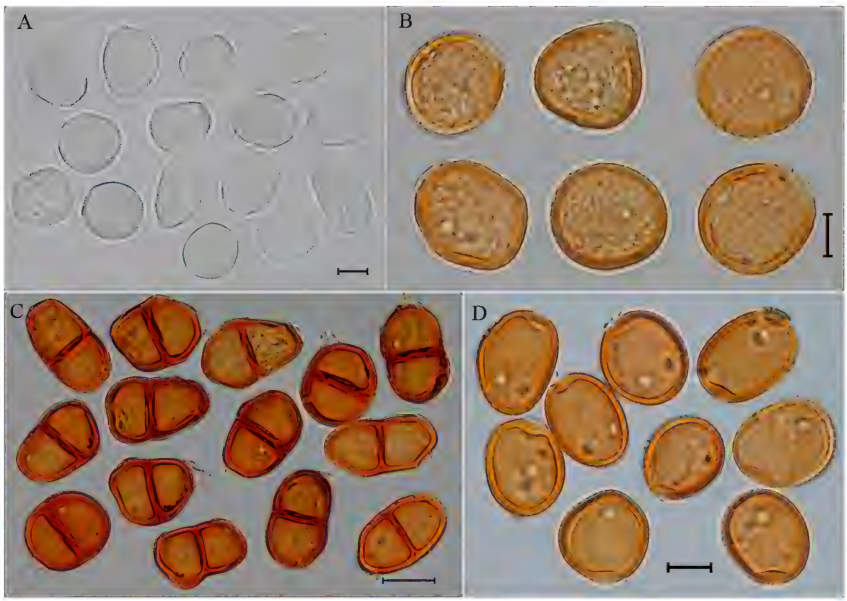

Plate 2. ***Puccinia crepidis-asadbarensis*** on *Crepis asadbarensis:* A. aeciospores; B. urediniospores; C. teliospores. ***Puccinia jaceae*** var. ***elbursensis*** on *Centaurea behen*: D. urediniospores. Scale bars: A–B, D=10 µm, C=20 µm.

Puccinia crepidis-asadbarensis Aliabadi & M. Abbasi, **sp. nov.** Plate 2A–C

Mycobank MB 564233

Differs from *Puccinia crepidis-pygmaeae* by its larger urediniospores and teliospores.

Type: On *Crepis asadbarensis* Bornm. ex Rech.f.: Iran, Tehran Prov., Central Alborz between Shemshak to Dizin, alt. 2800 m, I+II+III, 11 July 1994, leg. M.Abbasi (**holotype**, IRAN 14662 F).

Etymology: referring to the host species.

Spermogonia unknown. Aecia localized, in clusters; aeciospores 17.5–20 (–22.5) × 12.5–17.5 µm, mostly polygonal, wall hyaline, 1-1.5 µm thick, finely verrucose. Uredinia amphigenous, round, pulverulent, brown, rupturing the epidermis; urediniospores (20–)22.5–30(–32.5) × (17.5–)20–25(–27.5) µm,

globoid, ellipsoid or obovoid, wall echinulate, 1.5–2.5 µm thick, somewhat thicker at germ pores, germ pores 2(–3), equatorial. Telia amphigenous, round, pulverulent, black, rupturing the epidermis; teliospores (30–)32.5–40 (–42.5) × 22.5–27.5 µm, broadly ellipsoid or oblong, rounded at both ends, wall finely verrucose, 2.5 µm thick, germ pore of the upper cell apical or subapical, rarely close to the septum, and that of the lower cell close to the septum or equatorial.

Additional specimens examined: On *Crepis asadbarensis*: **IRAN**, **Tehran Prov.**, Central Alborz, Firoozkuh, Gaduk towards Doab, 20 km of Doab, Zagh-Bolagh Mountains, alt. 2300–2600 m, II+III, 30 June 1991, leg. F.Termeh & M.Mousavi (IRAN 14661 F); Shahrestanak, near Gilkola spring, alt. 2500 m, I+II+III, 12 August 1993, leg. M.Abbasi, J.Fatehi & O.Foitzik (IRAN 11174 F).

Comments — On the basis of the identification key published by Braun (1981), *P. crepidis-asadbarensis* is close to *P. crepidis-pygmaeae* but differs in having larger urediniospores (mostly >25 µm in diam.) and teliospores (32–40 µm long). *Puccinia crepidis-pygmaeae* urediniospores are mostly 20–25 µm long and teliospores 24–31 × 19–24 µm (Braun 1981). The host plant, *Crepis asadbarensis,* endemic to the Alborz area, belongs to section *Berinia* (Rechinger et al. 1977); no rust has previously been reported on plant species from this section in Iran.

Puccinia jaceae* var. *elbursensis Aliabadi & M. Abbasi, **var. nov.** Plate 2D

Mycobank MB 564234

Differs from *Puccinia jaceae* var *jaceae* by its urediniospores with larger smooth patches around the germ pores.

Type: On *Centaurea behen* L.: Iran, Tehran Prov., around Karaj, Gholgholak Spring, II+III, 22 May 1998, leg. M.Abbasi (**holotype**, IRAN 14667 F).

Etymology: referring to the mountain range near the type locality.

Spermogonia and aecia not seen. Uredinia amphigenous, round, pulverulent, cinnamon-brown, surrounded by the ruptured epidermis; urediniospores 25–32(–37.5) × 20–27.5 µm, mostly ellipsoid or globoid, wall echinulate, 1.5–2.5 µm thick, yellowish brown, germ pores 2(–3), supraequatorial, with smooth patches around the germ pores, up to 18 µm diam. Telia amphigenous, round, scattered, sometimes with a central sorus surrounded by an encircling group of sori (arranged in concentric circles), pulverulent, blackish brown; teliospores (35–)37.5–45(–52.5) × 22.5–27.5 µm, broadly ellipsoid, wall 2–3 µm thick, slightly thicker at pores, verrucose, sometimes verrucae arranged in more or less evident longitudinal rows, germ pore of the upper cell subapical and that of the lower cell approximately equatorial, germ pores covered by minute, verrucose papillae.

Additional specimens examined — On *Centaurea behen*: **IRAN**, **Tehran Prov.**, 25 km of NE of Tehran, Latian dam, II+III, August 1968, leg. M.Iranshahr (IRAN 14666 F);

Karaj-Chalus road, Kondor village, in an orchard, alt. 1800 m, II+III, 17 June 1997, leg. M.Abbasi et al. (IRAN 14668 F).

Comments — To judge from Braun (1981), *P. jaceae* is delimited from *P. hieracii* by teliospores with a mean width >25 µm and urediniospores with smooth patch <10 µm. This rust species is restricted to *Centaurea* spp. (Braun 1981). Interestingly, all three rust samples on *C. behen* we studied had teliospores with a mean width >25 µm (similar to *P. jaceae*) but urediniospores with smooth patches >10 µm (different from *P. jaceae*), leading us to describe the new variety, *P. jaceae* var. *elbursensis*.

Re-examination of the *P. jaceae* specimen on *Centaurea macrocephala* Puschk. ex Willd. (IRAN 13722 F) reported by Donyadoost-Chalan et al. (2009) showed urediniospores with smooth patches <10 µm, identifying it as *P. jaceae* var. *jaceae* in agreement with Braun (1981).

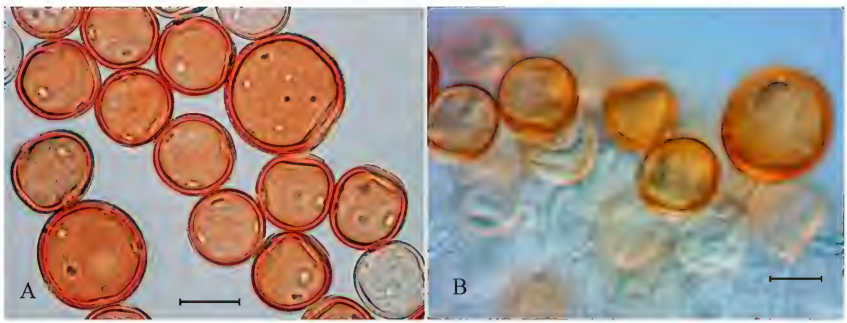

Plate 3. ***Puccinia punctiformis*** var. ***karajensis*** on *Cirsium arvense:* A. urediniospores; B. cross-section of uredinium. Scale bars = 20 µm.

Puccinia punctiformis* var. *karajensis Aliabadi & M. Abbasi, **var. nov.** Plate 3

Mycobank MB 564235

Differs from *Puccinia punctiformis* var. *punctiformis* by its dimorphic urediniospores.

Type: On *Cirsium arvense* (L.) Scop.: Iran, Tehran Prov., Karaj-Chalus road, 2 km S of Asara, around the river, alt.1750 m, O+II+III, 30 June 1999, leg. M.Abbasi et al. (**holotype**, IRAN 14659 F).

Etymology: referring to the general type locality.

Spermogonia crowded, amphigenous, in large clusters, honey–colored. Uredinioid aecia mostly hypophyllous and on the stems, systemic, scattered over the whole surface of the leaf, round to oblong, pulverulent, reddish brown; urediniospores of two types, normal urediniospores 23–31 × 23–27 µm, wall echinulate, 1.5–2 µm thick, mostly globoid or broadly ellipsoid, with (2–)3 equatorial germ pores; large urediniospores 33–37(–44) × 32–37(–44) µm, globoid, with 5–8(–10) scattered germ pores. Telia chiefly hypophyllous, rarely epiphyllous and on stems, round to oblong, pulverulent, sometimes covered

by the plant epidermis, black or brownish black; teliospores 34–40 × 21–26 µm, mostly ellipsoid to broadly ellipsoid or obovoid, rounded at both ends or slightly narrowed basally, wall 1.5–2.5 µm thick, chestnut-brown, scattered and finely verrucose, germ pore apical in upper cell, in lower cell close to the pedicel, pedicels short.

Comments — *Puccinia punctiformis* var. *karajensis* differs from *P. punctiformis* var. *punctiformis* in having dimorphic urediniospores with a second type of unusually large spores with 5–8 scattered germ pores. We observed cross-sections of uredinia with larger spores sitting on pedicels surrounded by normal urediniospores. Study of 12 Iranian *P. punctiformis* herbarium specimens from 5 different provinces (IRAN 12389, 9560, 11625, 11776, 4011, 8371, 4010, 6851, 1866, 13752, 15114, 15115 F) and specimens from Turkey (IRAN 6847 F) and Finland (IRAN 4482 F) showed no dimorphic urediniospores.

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Microfungi from Portugal: *Minimelanolocus manifestus* sp. nov. and *Vermiculariopsiella pediculata* comb. nov.

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Abstract —Two microfungi from a forest in Portugal are described and illustrated. The new species *Minimelanolocus manifestus* is distinguished by polyblastic, integrated, sympodial conidiogenous cells, and solitary, cymbiform, (2–)3-septate, smooth conidia with pale brown middle cells and subhyaline end cells. A new combination *Vermiculariopsiella pediculata* is proposed for *Gyrothrix pediculata*.

Key words — anamorphic fungi, systematics, leaf litter

During the Iberian mycobiota study's twenty-fifth mycological foray, held in "Lagoas de Bertiandos" (Ponte de Lima, Minho province) Portugal, two interesting and unusual anamorphic fungi were collected. Individual samples of plant material were placed in paper and plastic bags, taken to the laboratory, and treated according to Castañeda-Ruiz (2005). Mounts were prepared in polyvinyl alcohol-glycerol (8 g in 100 ml of water, plus 5 ml of glycerol) and measurements made at a magnification of ×1000. Photomicrographs were obtained with a Zeiss AXIO imager M1 microscope (Göttingen, Germany). The fungus was isolated into pure culture by transferring single conidia observed under a stereo microscope onto potato-carrot agar (PCA) in Petri dishes, then incubated at 25°C under daylight, 12 h alternating cycles of light/dark. Colour notations in parentheses are from Kornerup & Wanscher (1978). The species are described below.

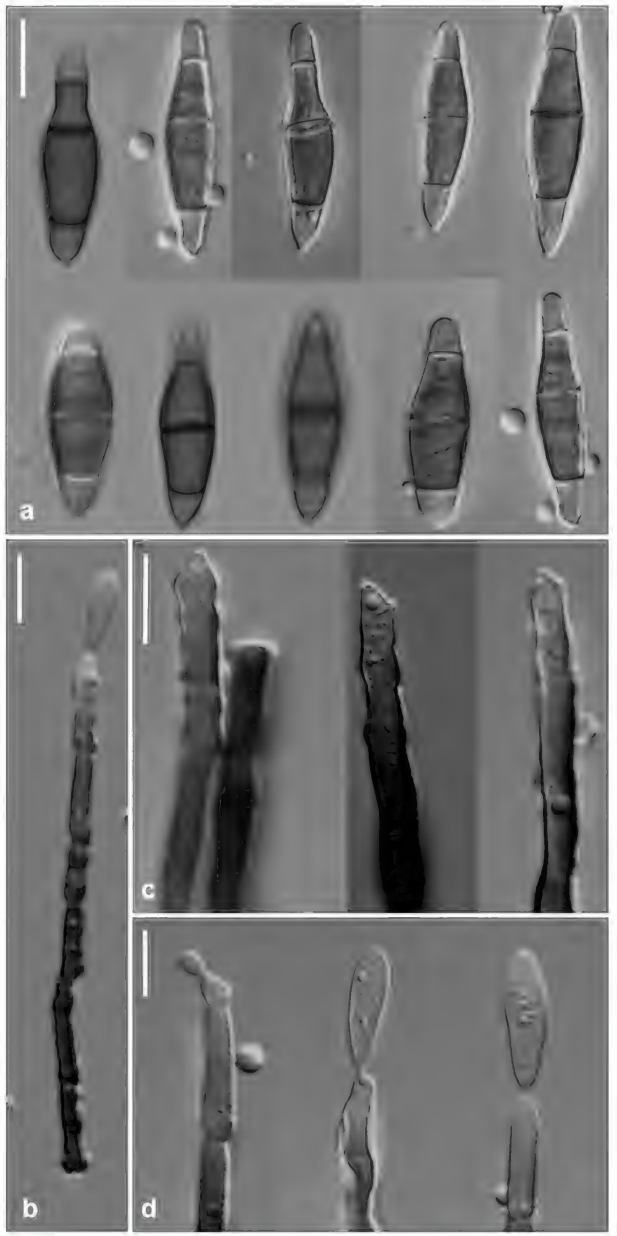

Fig 1. *Minimelanolocus manifestus* (ex holotype HAL 2450 F). a. Conidia. b. Conidiophore, conidiogenous cell and conidium. c. Conidiogenous cells. d. Conidiogenous cells and conidia. Scale bars = 10 µm.

Taxonomy

Minimelanolocus manifestus Hern.-Rest., R.F. Castañeda, Gené & Guarro, **anam. sp. nov.** Figs 1, 2

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Differs from *Minimelanolocus* spp. by conidia cymbiform to subfusiform, (2–)3-septate, with central cells pale brown and end cells subhyaline.

Type: Portugal, Minho province, "Lagoas de Bertiandos", 41°46′N 8°38′W, FMR 12182, on rotten leaf of unidentified plant, 9 November 2011, R.F. Castañeda, M. Hernández-Restrepo, J. Gené & J. Mariné-Gené (**Holotype**: HAL 2450 F).

Etymology: Latin, *manifestus*, meaning evident, clear manifest.

Colonies on the natural substratum, effuse, hairy, brown to dark brown. Mycelium superficial and immersed. Hyphae septate, branched, 2–4 µm diam., smooth, pale brown to brown. Conidiophores macronematous,

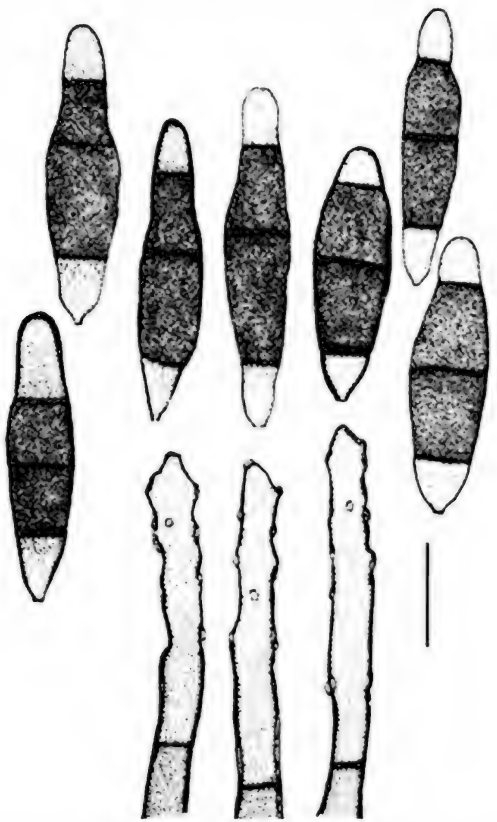

Fig 2. *Minimelanolocus manifestus* (ex holotype HAL 2450 F). Conidiogenous cells and conidia. Scale bar = 10 µm.

mononematous, erect, straight, sometimes slightly sinuate towards the apex, simple, 4–8-septate, smooth, 130–200 × 4–5 µm, brown at the base, elsewhere pale brown. Conidiogenous cells polyblastic, terminal, pale brown, 20–70 × 4–5 µm, integrated, indeterminate, with several sympodial proliferations bearing inconspicuous scars. Conidial secession schizolytic. Conidia solitary, smooth, dry, acropleurogenous, cymbiform to subfusiform, fimbrillate at the base, obtuse at the apex, (2–)3-septate, (21–)25–29 × 5–6 µm, with central cells pale brown and end cells subhyaline. Teleomorph unknown.

Note: Castañeda et al. (2001) established the genus *Minimelanolocus* with *M. navicularis* (R.F. Castañeda) R.F. Castañeda as type species to accommodate one new species and eleven combinations derived from *Pseudospiropes* M.B. Ellis, *Helminthosporium* Link, and *Belemnospora* P.M. Kirk. Subsequently, the following nine additional species were described: *M. camelliae* H.B. Fu & X.G. Zhang, *M. chimonanthi* Y.D. Zhang & X.G. Zhang, *M. endospermi* Jian Ma & X.G. Zhang, *M. linderae* Jian Ma & X.G. Zhang, *M. machili* K. Zhang & X.G. Zhang, *M. magnoliae* K. Zhang & X.G. Zhang, *M. mori* Jian Ma & X.G. Zhang, *M. olivaceus* R.F. Castañeda & Guarro, and *M. pterocarpi* Jian Ma & X.G. Zhang (Castañeda et al. 2003, Ma et al. 2008, 2011, Zhang et al. 2009, 2010). Among the known species, *M. manifestus* most closely resembles *M. linderae* and *M. navicularis*, but *M. linderae* has ellipsoidal or cylindrical conidia, 26–35 × 7–9 µm, which are mostly 4-septate and rarely 3-septate, while *M. navicularis* possesses navicular to sub-rhombic conidia, 20–25 × 6–8 µm, which are (2–)3(–5)-septate, with brown to dark brown central cells and subhyaline end cells. All three species can be easily differentiated.

Vermiculariopsiella pediculata (J.L. Cunn.) Hern.-Rest., R.F. Castañeda, Gené & Guarro, **comb. nov.** Figs 3–5

MycoBank MB800053

≡ *Gyrothrix pediculata* J.L. Cunn., Mycologia 66(1): 123 (1974).

Anamorphic fungus. Colonies on the natural substratum effuse, blister-shaped to sporodochial, hairy, whitish, amphigenous. Mycelium superficial and immersed. Hyphae septate, frequently anastomosing, 1–3 µm diam. Setae erect, cylindrical, 110–220 × 4–6 µm, but wider, 5–9 µm diam at the base, with alternate, irregular to imperfect dichotomous branches, the branches being attenuate, coiled to whip-like, curved, septate, smooth, brown below, and pale brown at the apex. Conidiophores comprised solely of conidiogenous cells. Conidiogenous cells monophialidic, discrete, ampulliform to subulate, sometimes very close packed forming a cespitose layer, pale brown at the base, subhyaline at the apex, 14–15 × 3–4 µm, with a distinct, narrow, refractive collarette. Conidia asymmetrical, sub-fusiform to navicular or sub-oblong, attenuate, somewhat apiculate, curved at the apex, tumid, somewhat irregular,

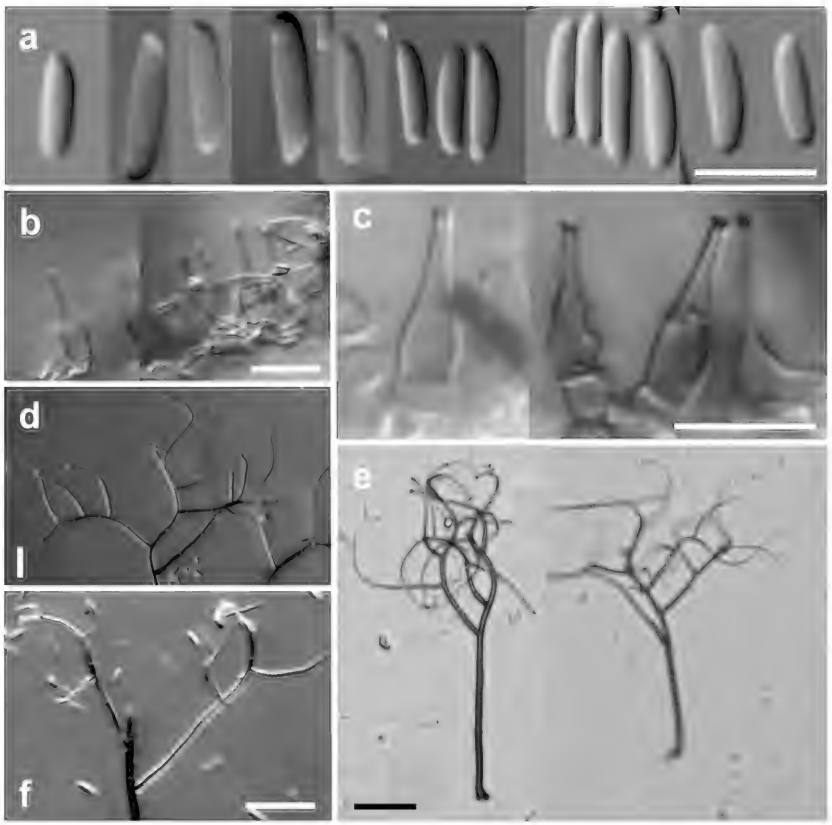

Fig 3. *Vermiculariopsiella pediculata* (ex nature HAL 2447 F). a. Conidia. b, c. Conidiogenous cells. d-f. Setae. Scale bars = 10 μm.

eccentrically and inconspicuously fimbrillate at the base, unicellular, hyaline, smooth, 5–9 × 2 μm, produced seriately and obliquely from the conidiogenous loci, forming a white cirrhus or pseudochains in snow-like masses.

Specimen examined: **PORTUGAL**, **Minho province**, "Lagoas de Bertiandos", 41°46′N 8°38′W, FMR 12187, on rotten leaf of unidentified plant, 9 November 2011, R.F. Castañeda, M. Hernández-Restrepo, J. Gené & J. Mariné-Gené (HAL 2447 F).

Colonies on PCA, attaining 21–30 mm after 7 days at 25°C, floccose, raw umber (5F8) with yellowish white (4A2) and numerous white pustules somewhat pulvinate formed by conidial cirrhus. Setae similar to those on the natural substratum, 100–149 × 4–6 μm. Hyphae septate, subhyaline to very pale brown, 1–2 μm diam, smooth. Conidiophores comprised solely of conidiogenous cells. Conidiogenous cells monophialidic, subulate to ampulliform, slightly sinuate or curved, very pale brown at the base, hyaline

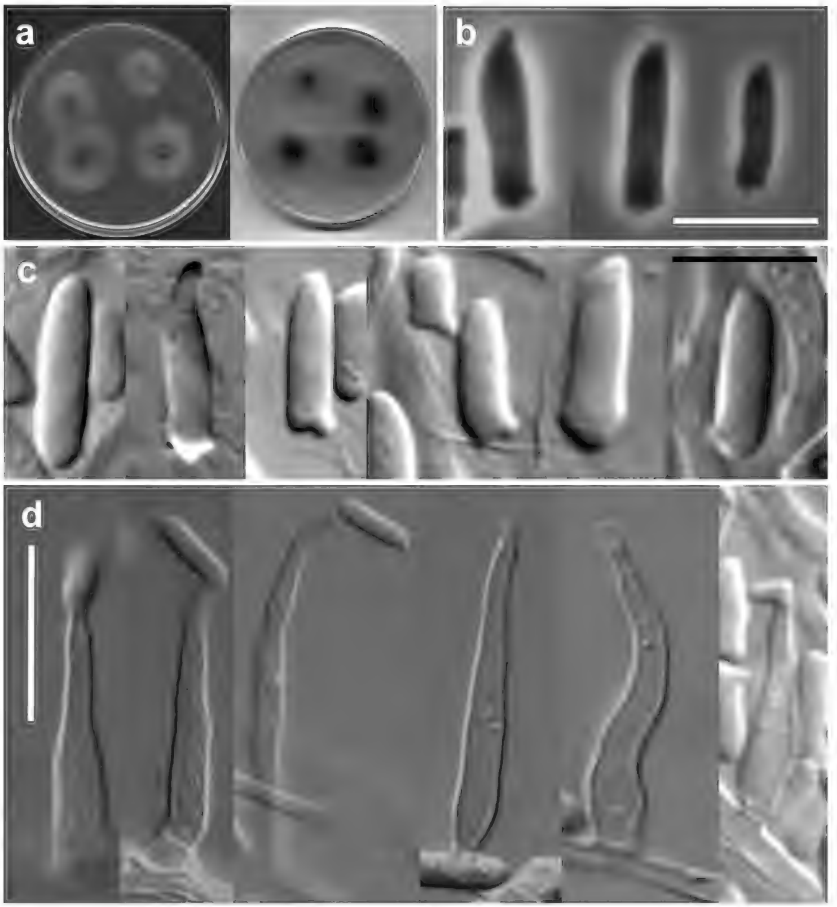

Fig 4. *Vermiculariopsiella pediculata* (ex culture HAL 2451 F). a. Colonies on PCA (obverse and reverse, respectively) at 25°C after 7 days. b, c. Conidia. d. Conidiogenous cells. Scale bars = 10 µm.

towards the apex, 19–28 × 3–4 µm. Conidia asymmetrical obclavate to long obovoid, oblong or irregular, obtuse, slightly curved or subuncinate at the apex, inflated, tumid, eccentrically fimbrillate at the base, hyaline, 19–28 × 3–4 µm, smooth-walled, produced axially at the conidiogenous cell apex in a long white cirrhus or pseudocatenate.

Cultures deposited: (ex HAL 2447 F) HAL 2451 F, CBS 132484, FMR 12187.

Note: Nakagiri & Ito (1991) illustrated the conidiogenous cells and conidium ontogeny from *Gyrothrix circinata* (Berk. & M.A. Curtis) S. Hughes and regarded conidial production as "phialides type"; the authors interpreted the whorled arrangement of conidia at the conidiogenous cell apex as resulting

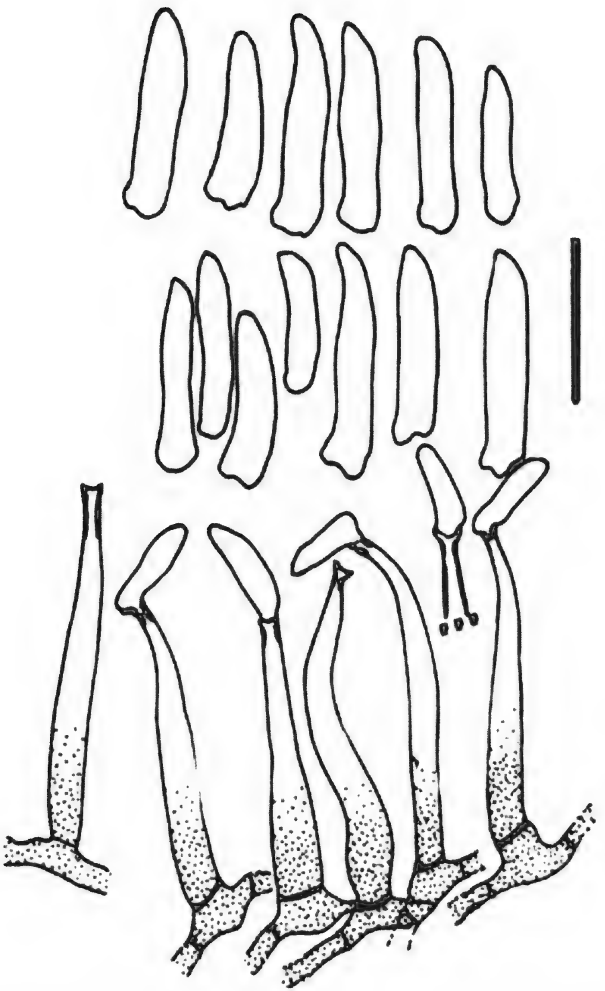

Fig 5. *Vermiculariopsiella pediculata* (ex culture on PCA, HAL 2451 F). Conidia and conidiogenous cells. Scale bar = 10 µm.

from mucilaginous material produced by and accumulated at the tip of each conidiogenous cell. Crous et al. (1996), working with *Gyrothrix verticiclada* (Goid.) S. Hughes & Piroz., illustrated inconspicuous annellations at the conidiogenous cell apices produced after enteroblastic percurrent proliferations. The aggregated conidia forming a whitish, more or less uniform layer at the base of each seta is clearly the result of a conidial ontogeny with successive enteroblastic percurrent or sympodial proliferations occurring near the apex

of each conidiogenous cell, but the number of conidia produced by this way is apparently limited and can be differentiated from the conidia produced from a "phialide" as described by Kirk et al. (2008).

Wu et al. (1997) suggested that conidiogenous cells in *Ceratocladium*, *Circinotrichum,* and *Gyrothrix* are not typical "phialides". Cunningham (1974) described and illustrated the conidiogenous events on *Gyrothrix pediculata* from cultures on corn meal agar. This pattern of ontogeny was classified as conidial development type 18 (conidium ontogeny holoblastic, delimitation by 1 septum, schizolytic secession, maturation by diffuse wall-building, percurrent and sympodial enteroblastic conidiogenous cell proliferation below the previous locus and delimiting septum, the second and subsequent conidia formed from proliferations, but with no observed reduction in length of the conidiogenous cells). Setose conidiomata with branched setae and similar conidial ontogeny can be found in *Vermiculariopsiella* Bender [*V. cornuta* (V. Rao & de Hoog) Nawawi, Kuthub. & B. Sutton, *V. cubensis* (R.F. Castañeda) Nawawi, Kuthub. & B. Sutton, *V. microsperma* (Höhn.) R.F. Castañeda & W.B. Kendr., and *V. ramosa* (B. Sutton) Nawawi, Kuthub. & B. Sutton]. As "phialides" were described for the conidiogenous cells in *Vermiculariopsiella* by Morgan-Jones et al. (1972), Nawawi et al. (1990) and Keshavaprasad et al. (2003), the transfer of *G. pediculata* to *Vermiculariopsiella* is clearly supported and agrees with the generic concept.

Acknowledgments

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Geastrum species of the La Rioja province, Argentina

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Abstract—A survey of *Geastrum* species from arid lands in the La Rioja province (northwestern Argentina) recovered fourteen species previously unreported from the province: *G. arenarium*, *G. campestre* var. *famatinum*, *G. corollinum*, *G. fimbriatum*, *G. floriforme*, *G. glaucescens*, *G. hieronymi*, *G.* aff. *lloydianum*, *G. pampeanum*, *G. pampeanum* var. *pallidum*, *G. parvistriatum*, *G. rufescens*, *G. saccatum*, and *G. schmidelii*. Of these, new records are *G. corollinum* for Argentina, *G. arenarium* for South America, and *G. parvistriatum* for the Americas, and *G. campestre* var. *famatinum* is proposed as a new variety. Each taxon is described, and photographs of basidiomes and SEM micrographs illustrating spores are provided for selected taxa.

Key words— *Geastrales*, mycobiota, *Phallomycetidae*, taxonomy

Introduction

The genus *Geastrum* (earthstars) is characterized by subspherical to spherical basidiomes having a central axis (columella) surrounded by a mass of fertile tissue (gleba) contained within an inner (endoperidium) and outer (exoperidium) layer. Spores are liberated through a single apical pore (in the endoperidium) surrounded by a highly variable structure called the peristome. At maturity, the exoperidium splits into a variable number of rays, which give *Geastrum* basidiomes their characteristic star-shape. These exoperidial rays serve to protect the endoperidial body and facilitate spore dispersal. With their unique morphology, *Geastrum* species represent the evolution of one of the more specialized basidiome forms found among the gasteroid fungi.

The present paper focuses on *Geastrum* species that were collected during survey of gasteroid fungi of the La Rioja province of northwestern Argentina. La Rioja belongs to the Monte biogeographical province, which is defined as a xeric biome (80–200 mm annual rainfall) based on its vegetation and

physiognomy (Roig 1998; Roig-Juñent 2001). The predominant vegetation is a shrub steppe, which is a characteristic endemic flora mainly represented by vascular plant species in the *Zygophyllaceae* (e.g., *Larrea*, *Bulnesia*, and *Plectrocarpa*). Each *Geastrum* taxon recovered in the survey is described and discussed, and selected taxa are illustrated.

Materials & methods

This work is based on specimens collected by L. Papinutti and G. Rolón and deposited in the University of Buenos Aires Herbarium (BAFC). Capillitial threads and spores were observed under light (mounted in KOH 3% w/v) or scanning electron (Zeiss DSM 982 Gemini) microscopy. At least 20 measurements were made to estimate the size of microscopic and macroscopic elements. Macroscopic measures were made on dry basidiomes. All spore measurements citing the diameter include the ornamentation. Colors were described in well-preserved dry specimens and indicated following Maerz & Paul (1930). Bates (2004), Dominguez de Toledo (1989), Sunhede (1989), Soto & Wright (2000), Ponce de Leon (1968), Calonge (1998) and Zamora & Calonge (2007) were used as standard references in the determination of collections.

Taxonomy

Geastrum arenarium Lloyd, *Geastrae:* 28 (1902, as" *Geaster arenarius*"). Figs 1c, 2a

Mature basidiomes 20–30 mm wide; exoperidium arched, splitting in 10–12 subequal arching, revolute, hygroscopic rays; mycelial layer encrusting fine particles of debris and sand; fibrous layer creamy white (2A1); pseudoparenchymatous layer whitish to pallid brown (14C7) when immature, rimulose in age; endoperidial body 5–12 mm wide almost sessile, globose, slightly pruinose, pallid brown (14C7); apophysis reduced; peristome fibrillose, conical, delimited, darker, concolorous, or lighter than the surrounding endoperidium; gleba brown (7H10), cottony.

Spores globose, 2.5–3.7 µm wide, verrucose, with a short apiculus that is difficult to discern due to the dense covering of mammiform verrucae.

Specimen examined—**ARGENTINA. La Rioja:** Miranda, 28°28′33.68″S 67°42′27.55″W, gregarious on abundant leaf litter under *Prosopis* sp. (*Mimosaceae*), 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51925).

Comments—Although most basidiomes exhibited hygroscopic rays, sub-hygroscopic rays were also observed. A very short stalk was observed in some dry specimens similar to North American material described by Bates (2004; referred to by the author as "± sessile"), while other specimens were entirely sessile. *Geastrum arenarium* may be confused with other hygroscopic species, *G. corollinum* and *G. floriforme*, from La Rioja. However, these taxa have larger spores than *G. arenarium* and a mycelial layer not strongly encrusted with sand and debris. This is the first record of this species for Latin America.

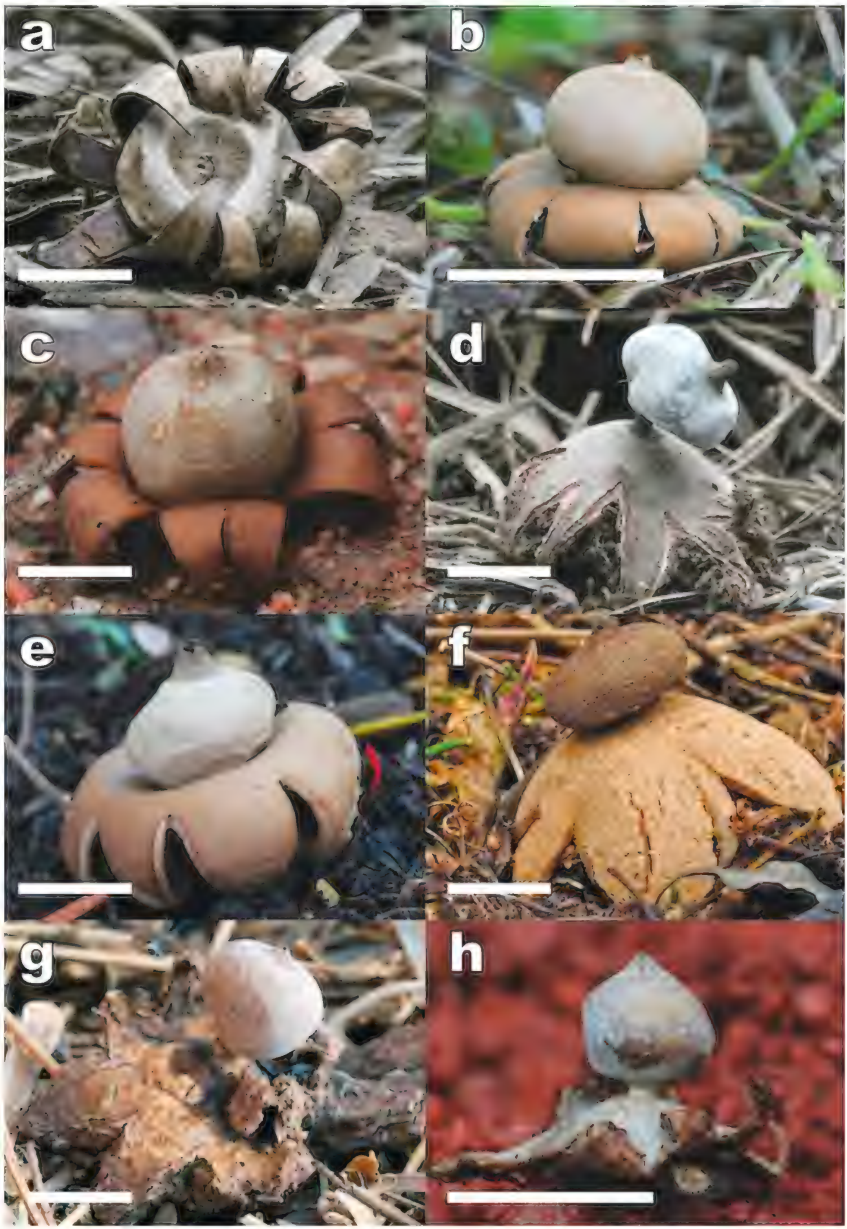

FIGURE. 1: a. *G. corollinum*. b. *G. floriforme*. c. *G. arenarium*. d. *G. parvistriatum*. e. *G. saccatum*. f. *G. hieronymi*. g. *G. campestre* var. *famatinum*. h. *G. glaucescens*. Scale bars = 1 cm.

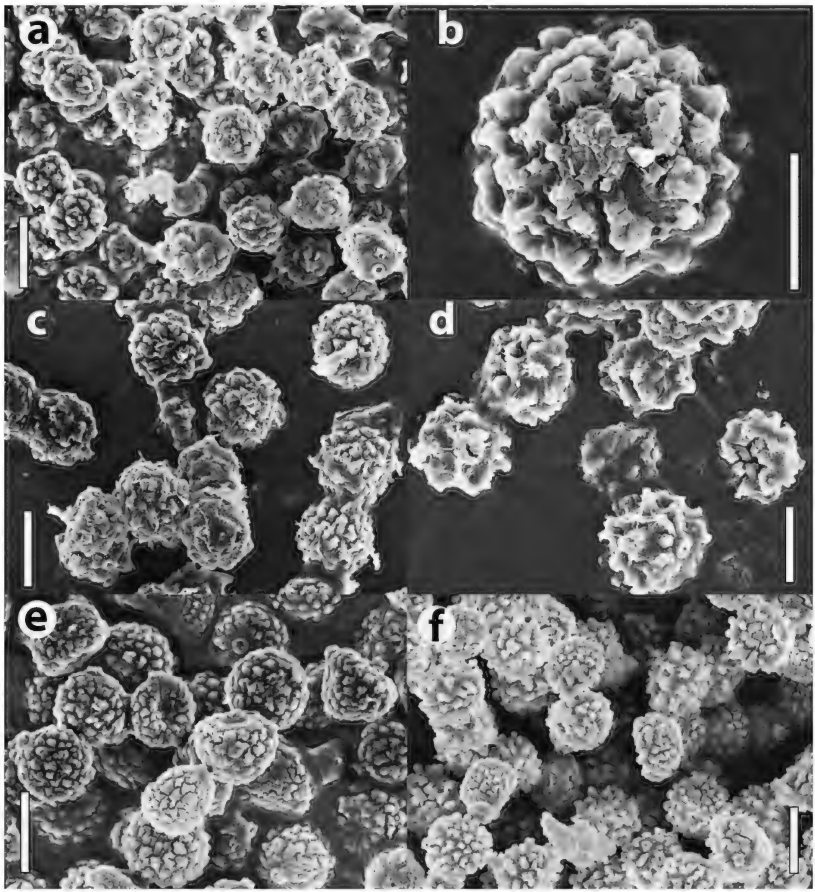

FIGURE. 2: SEM micrographs of spores. a. *G. arenarium*. b. *G. hieronymi*. c. *G. glaucescens*. d. *G. parvistriatum*. e. *G.* aff. *lloydianum*. f. *G. pampeanum* var. *pampeanum*. Scale bars: a, c–f = 4 μm; b = 2 μm.

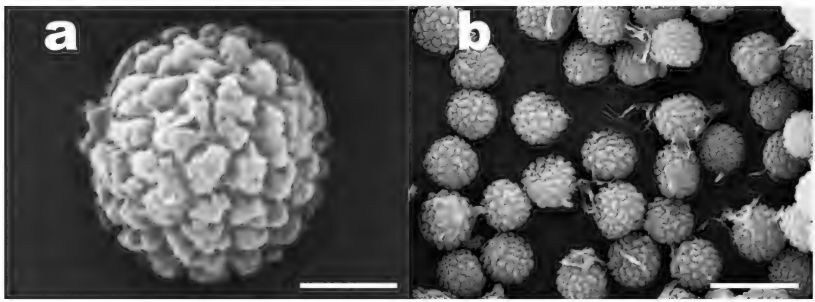

FIGURE. 3: SEM micrographs of *G. campestre* var. *famatinum* spores.
Scale bars: a = 1 μm; b = 5 μm.

Geastrum campestre var. ***famatinum*** Kuhar & Papinutti, **var. nov.** FIGS 1g, 3

MycoBank MB 563048

Differt a typo Geastri campestris magnitudine sporarum valde minore.

TYPE: Argentina. La Rioja: Famatina Department, Pituil, 28°34′47.94″S 67°27′59.42″W under *Prosopis* sp., 27.III.2008, leg. L. Papinutti & G. Rolón, (**holotype**,, BAFC 51926).

ETYMOLOGY: from the name of the geographic department of the type locality.

Expanded basidiomes 20–35 mm wide; exoperidium arched, splitting in 9–12 subequal rays, revolute and curving under the endoperidial body, weakly hygroscopic; mycelial layer encrusted with abundant debris, peeling off toward the ray tips with age but persisting in the rest of the endoperidium; fibrous layer light colored (12G7) and smooth; pseudoparenchymatous layer dirty dark brown (8E8), peeling off of the fibrous layer in an irregular manner; endoperidial body 10–18 mm wide globose with a very short stalk, verrucose and slightly pruinose, grey (14A3), laterally compressed in some specimens; apophysis visible; peristome definite, plicate, conic, concolorous with the surrounding endoperidium; gleba dark brown (8A11), cottony.

Spores globose, 3.5–4.2 µm wide, verrucose.

COMMENTS—This collection agrees very precisely with published descriptions of *G. campestre*, but the spores are much smaller than those measured by Sunhede (1989; 6.5–8 µm), Ponce de León (1968; 5–6 µm) and Bates (2004; 6.4–8 µm). Domínguez de Toledo (1989), in her prospection of Córdoba and La Pampa provinces (Argentina), reported some collections of *G. campestre* having, on average, half the spore size than is typical for this species. Soto & Wright (2000), who described very similar basidiomes, treated them as *G. ambiguum* due to the smaller spore size. Sunhede (1989), who carefully studied type material of *G. ambiguum* deposited at Kew, concluded that it is a synonym of *G. campestre*. Another similar species is *G. smithii*, which differs from *G. campestre* in having spores more densely covered with shorter verrucae, a rimose pseudoparenchymatous layer, and a peristome surrounded by a depressed area. The granulose endoperidium is a striking characteristic that clearly differentiates *G. campestre* from other similar species. Since the La Rioja collection fits the macromorphological description of *G. campestre* reported in Sunhede (1989) but with considerably smaller spores, we propose this as a new variety.

Geastrum corollinum (Batsch) Hollós, Gasteromyc. Ungarns: 62 (1904, as "*Geaster corollinus*"). FIG. 1a

Basidiomes small to very small 6–19 mm wide; exoperidium saccate to slightly arched, very hard in old specimens, splitting into 8–11 strongly hygroscopic rays that cover the endoperidial body; mycelial layer encrusting debris but soon peeling off; fibrous layer whitish grey (5A8); pseudoparenchymatous layer very

persistent, turning brown to dark brown (8L6) in old specimens; endoperidial body sessile, globose, greyish beige (12B4); peristome fibrillose, delimited; mature gleba brown (7A11).

Spores globose, 4.0–5.0 µm wide, verrucose, with some verrucae coalescing at their apices; apiculus distinct.

Specimens examined—**ARGENTINA. La Rioja**: Santa Cruz, 28°28′35.11″S 67°41′54.45″W, gregarious on abundant leaf litter under *Prosopis* sp., 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51927); 28°28′42.14″S 67°42′32.31″W, among litter under *Prosopis* sp., 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51928); 28°28′42.15″S 67°42′49.79″W, on abundant leafy litter under *Prosopis* sp. near a dry river, 28.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51929).

Comments— Although fruit body size is homogeneous within each of our collections, the variation among different collections is high. Sunhede (1989) clearly illustrates the variability of this character. *Geastrum corollinum* and *G. glaucescens* are the most frequent species found in our survey of this province. This is the first report of *G. corollinum* for Argentina.

Geastrum fimbriatum Fr., Syst. Mycol. 3(1): 16 (1829, as "*Geaster fimbriatus*").

Basidiomes small to medium sized 20–25 mm wide; exoperidium often saccate, splitting from about the middle in 6–10 non-hygroscopic to sub-hygroscopic rays with light margins; mycelial layer encrusting debris but peeling off in some specimens; fibrous layer light beige (4C7) on both sides in fresh specimens, becoming denuded and whitish in older ones; pseudoparenchymatous layer becoming brownish in age (7A11); endoperidial body 9–11 mm wide greyish brown (8C11), sessile, globose to depressed globose; peristome fibrillose, not delimited, sometimes slightly protruding; gleba brown (8L6).

Spores globose, 3.5–4.0 µm wide, verrucose with ≤0.4 µm long columnar verrucae; capillitial hyphae thick walled.

Specimen examined—**ARGENTINA. La Rioja**: Guanchín, 29°09′40.98″S 67°39′40.37″W, gregarious, on finely particulated debris of diverse plant species, 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51930).

Comments—*Geastrum fimbriatum,* one of the most widely distributed species in the genus, has been cited in Europe (Sunhede 1989; Calonge 1998), North America (Bates 2004), and Latin America (Leite et al. 2007; Pereira et al. 2009; Calonge et al. 2005), including Argentina's Buenos Aires province (Soto & Wright 2000). This is the first record for La Rioja province.

Geastrum floriforme Vittad., Monog. Lycoperd.: 23 (1842, as "*Geaster floriformis*").

Fig. 1b

Basidiomes small to very small, 5–10 mm wide, hypogeous prior to opening; exoperidium saccate to slightly arched when fresh, very hard when dry, splitting in about 6–11 strongly hygroscopic rays, these covering the

endoperidial body in the dry state; mycelial layer encrusting debris but peeling off early in the development of the basidiome; fibrous layer greyish (4A1); pseudoparenchymatous layer very persistent, turning brown (8L6) to dark brown in old specimens; endoperidial body 3-6 mm wide, sessile light brown (14E7); peristome fibrillose not delimited; apophysis absent; gleba brown (7A11).

Spores globose, 5.0–5.5 µm wide, verrucose with short irregularly shaped verrucae; apiculus distinct.

Specimen examined— **ARGENTINA. La Rioja:** Santa Cruz, 29°20′45.54″S 67°42′22.16″W, on *Larrea divaricata* and *Baccharis* sp. leaf litter, 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51931).

Comments—In Argentina *G. floriforme* was documented from Córdoba and Catamarca provinces (Domínguez de Toledo, 1989) and Buenos Aires province (Soto & Wright 2000). This is a first record for La Rioja.

Geastrum glaucescens Speg., An. Mus. Nac. Bs. As. 23: 14 (1912, as "*Geaster*"). Figs 1h, 2c

Mature basidiomes 11–24 mm wide; exoperidium weakly arched, splitting in 9–10 subequal involute sub-hygroscopic rays; mycelial layer encrusted with fine debris, fibrous layer whitish; pseudoparenchymatous layer greyish beige in age (5A8), breaking tangentially at the base of the rays; pseudoparenchymatous layer and endoperidial body covered with abundant persistent white mesoperidial matter; endoperidial body 5–10 mm wide, stalked, globose, surface slightly pruinose, concolorous with the inner exoperidial surface (7A10 to 6A7); stalk white, 1 mm long; apophysis absent; peristome plicate, conical, delimited, concolorous to dark brown at the very tip; gleba brown (7H12), cottony.

Spores globose, 3.5–4.5 µm wide, with a short apiculus that is difficult to discern due to the covering of short mammiform verrucae.

Specimens examined— **ARGENTINA. La Rioja:** Santa Cruz, 29°20′45.54″S 67°42′22.16″W, solitary, on leaf litter of *Larrea divaricata* and *Baccharis* sp., 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51932); Chilecito, 29°09′11.20″S 67°29′34.18″W, abundant, gregarious, on leaf and particulated debris under *Prosopis* sp., 15.IV.2009, leg. L. Papinutti & G. Rolón (BAFC 51933); Chamical, 30°20′18.74″S 66°20′23.79″W, on leaf litter of *L. divaricata*, 2.IV.2007, leg. L. Papinutti & G. Rolón (BAFC 51934); 30°22′21.10″S 66°17′03.90″W, on leaf litter of *Prosopis* sp., 2.IV.2007, leg. L. Papinutti & G. Rolón (BAFC 51935).

Comments— As Soto & Wright (2000) stated, *G. glaucescens* resembles small basidiomes of *G. striatum*; however, that species lacks the ring-like apophysis and has larger distinctly ornamented spores. Another similar species found in La Rioja is *G. parvistriatum,* which can be distinguished by larger basidiome size, larger spores with a less densely distributed ornamentation, and a subglobose, flattened, or ovoid endoperidial body. The *G. parvistriatum* stipe is

much longer and darker than in *G. glaucescens*. *Geastrum minimum* also shows similarities but differs from *G. glaucescens* in its a strongly arched exoperidium, fibrillose peristome, less pruinose endoperidial surface, conspicuous apophysis, and larger spore size. This is the first record of *G. glaucescens* from La Rioja, although Spegazzini's type collection was collected in Buenos Aires province.

Geastrum hieronymi Henn., Hedwigia 36: 211 (1897, as "*Geaster*"). FIGS 1f, 2b

Basidiomes medium sized 30–70 mm wide when fully open; exoperidium splitting from the middle (or deeper in some specimens) into mostly 6–8 acute rays, sometimes revolute, but not hygroscopic; mycelial layer persistent, brown, encrusting debris; fibrous layer light beige (11B3) when denuded; pseudoparenchymatous layer dark brown when dry (8H7); endoperidial body 20–30 mm wide, dirty bronze grey (15C8), subglobose, with a short stalk, asperate; apophysis prominent; peristome not delimited, fibrillose; gleba bordeaux (8L12).

Spores globose, 3.5–4.5 µm wide, verruculose.

SPECIMEN EXAMINED— **ARGENTINA. LA RIOJA**: Miranda, 29°20′51″S 67°42′22.16″W, on abundant leaf litter and twigs of *Prosopis* sp., 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51936).

COMMENTS—This is the first report of *G. hieronymi* from La Rioja.

Geastrum aff. ***lloydianum*** Rick, Brotéria 5: 27 (1906, as "*Geaster lloydianus*"). FIG. 2e

Basidiomes 35–40 mm wide; exoperidium splitting into 7–9 acute subequal rays, not hygroscopic; mycelial layer encrusted with sand; fibrous layer ochraceous (14G8); pseudoparenchymatous layer umber (8H12), cracking and seceding in places; endoperidial body 15–20 mm wide, subpedicellate or almost sessile, light beige (5A9), depressed globose, distinctly pitted and roughened but not truly warted or tomentose; apophysis absent; peristome sulcate, conical, acute, concolorous or darker than the endoperidium; gleba umber (7H11).

Spores globose, 3.0–4.0 µm wide, dark brown, finely verruculose.

SPECIMEN EXAMINED— **ARGENTINA. LA RIOJA**: Sañogasta, 29°18′43.88″S 67°35′37.95″W, on soil with scarce organic matter, 25.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51937).

COMMENTS— Our spores agree in size with the cited descriptions, but differ in the type of ornamentation illustrated for this species by Coker & Couch (1928), which showed sparse minute verrucae. We regard this collection as *Geastrum* aff. *lloydianum*, but SEM images of the spores from the type material are needed to clarify this determination. Another key character for this species is the dark endoperidial body (Ponce de Leon 1968); but a recent report of *G. lloydianum* with a yellowish brown endoperidial surface (Trierveiler-Pereira et al. 2011) suggests that this character can be highly variable.

Geastrum pampeanum Speg., Anal. Mus. Nac. Bs. As. 6: 192 (1899, as "*Geaster pampeanus*") var. ***pampeanum.*** FIG. 2f

Basidiome 25 mm wide, saccate; exoperidium membranaceous, delicate, splitting in 5 rays, these helicoidally twisted at tips in the dry state and partially covering the endoperidial body; mycelial layer sand colored, not encrusted with debris; fibrous layer light beige (12C5); pseudoparenchymatous layer dark hazel brown (8H12); endoperidial body 16 mm wide, sessile, hazel brown at maturity (14B7); peristome fibrillose, delimited, concolorous with the surrounding endoperidial body; apophysis absent; gleba dark brown (8L11).

Spores globose, 4.5–5.5 µm wide, verrucose with regular long (≤1 µm) columnar verrucae.

SPECIMEN EXAMINED— **ARGENTINA. LA RIOJA:** Chilecito, 29°09′11.20″S 67°29′34.18″W, solitary, on leaf and particulate debris under *Prosopis* sp., 20.II.2006, leg. L. Papinutti (BAFC 51939).

COMMENTS— This is the first record of this variety from La Rioja province.

Geastrum pampeanum var. ***pallidum*** Speg., Anal. Mus. Nac. Bs. As. 6: 192 (1899, as "*Geaster pampeanus* var. *pallida*").

Mature basidiomes 12–29 mm wide, saccate; exoperidium non-hygroscopic splitting into about 5–10 rays; mycelial layer, slightly encrusted with fine debris; fibrous layer creamy white (2A1); pseudoparenchymatous layer dark beige (8E12); endoperidial body 6-10 mm wide, sessile, brown at maturity (7C10); peristome fibrillose, delimited, slightly conic but depressed in dry state, concolorous with the surrounding endoperidial body; apophysis absent; gleba dark beige (8C11).

Spores globose, 3.2–4.5 µm wide, verrucose with conical to columnar verrucae.

SPECIMEN EXAMINED— **ARGENTINA. LA RIOJA:** Chilecito, 29°09′11.20″S 67°29′34.18″W, gregarious, on leaf and particulate debris under *Prosopis* sp., 20.II.2006, leg. L. Papinutti (BAFC 51938).

COMMENTS—This is the first record of this variety from La Rioja province.

Geastrum parvistriatum J.C. Zamora & Calonge, Bol. Soc. Micol. Madrid 31: 140 (2007). FIGS 1d, 2d

Mature basidiomes 20–40 mm wide; exoperidium non-hygroscopic, arched, splitting into 8–11 subequal, acute rays; mycelial layer encrusted with abundant debris; fibrous layer sand colored (12D6); pseudoparenchymatous layer brown (7C9), smooth, peeling off to expose the fibrous layer; endoperidial body 8–15 mm wide, stalked, grey to lead (7C7), smooth and always pruinose; stalk 1.5–2.5 mm high, dark brown; apophysis visible in some basidiomes; peristome conical, plicate–sulcate, well delimited and darker than the endoperidium; gleba brown (8C10).

Spores globose, 4.3–5.7 µm wide, verrucose with long verrucae that are frequently coalescent at their apices.

Specimen examined— **ARGENTINA. La Rioja:** Santa Cruz, 28°28′35.11″S 67°41′54.45″W, on abundant *Prosopis* sp. leaf litter, 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51940).

Comments—There are no differences between the accurate description of Zamora & Calonge (2007) and our material. Our collections were also made on sandy soil, as were the Spanish type material. This is the first report of *G. parvistriatum* from the Americas, although *G. striatum* (and perhaps *G. schmidelii*) specimens there should be re-evaluated in light of Zamora & Calonge (2007).

Geastrum rufescens Pers., Syn. Meth. Fung.: 134 (1801).

Basidiome medium sized, 25 mm wide; exoperidium non-hygroscopic, arched to saccate, splitting from the middle into 8 rays with light margins; mycelial layer encrusted with abundant debris; fibrous layer whitish to pale beige (13D5) only visible in the denuded margins of the rays; pseudoparenchymatous layer becoming brownish in age (14I9); endoperidial body 10 mm wide, globose to depressed globose, light brown (14E6) when dry; stalked, apophysis present, peristome fibrillose, not delimited, neither protruding nor thickened; gleba dark brown (8L6).

Spores globose, 3.5–5.0(–6.0) µm wide, verrucose with long columnar verrucae; capillitial hyphae thick walled.

Specimen examined— **ARGENTINA. La Rioja:** Chilecito, 29°09′11.20″S 67°29′34.18″W, on abundant *Prosopis* sp. leaf litter under *Ephedra triandra*, 21.II.2006, leg. L. Papinutti & G. Rolón (BAFC 51941).

Comments—Although our collection contains just one specimen, the perfectly preserved basidiome exactly fits the descriptions cited above. Soto & Wright (2000) have already reported *G. rufescens* from Buenos Aires province, and this is the first report of the species from La Rioja.

Geastrum saccatum Fr., Syst. Mycol. 3(1): 16. (1828, as "*Geaster saccatus*"). Fig. 1e

Basidiomes typically small though some are medium sized, 19–31 mm wide; exoperidium splitting to about the middle into 6–8(–12) rays of variable shape, often recurved under the whole basidiome; mycelial layer whitish when not covered with debris; fibrous layer beige (14K9); pseudoparenchymatous layer at first white to grey, but soon turning beige to almost dark brown (8H10), relatively thick, papery, sometimes with longitudinal cracks in older specimens, peeling off at the margins with age; endoperidial body (3.5–)5–20(–24) mm wide, globose to depressed globose, sessile or subsessile, pale grey to light beige (14A5) smooth; apophysis lacking; peristome finely fibrillose, mammiform

to almost flat, delimited, concolorous or darker than the endoperidium and sometimes surrounded by a light colored zone; gleba brown (8H9).

Spores globose, 4.5–6.0 µm wide, ornamented with short columnar verrucae (some apically coalescent).

Specimens examined— **ARGENTINA. La Rioja**: Miranda, 29°20′45.54″S 67°42′22.16″W, on *Prosopis* sp. leaf litter, 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51942, 51944); Sañogasta, 29°18′43.88″S 67°35′37.95″W, on finely particulated debris, 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51943).

Comments— Although collected in the same locality, the endoperidium color differs in *G. saccatum* BAFC 51944 and BAFC 51942. Nevertheless, Sunhede (1989) described this character as highly variable in this species. This is the first record of *G. saccatum* from La Rioja province.

Geastrum schmidelii Vittad., Monog. Lycoperd.: 13. (1842, as "*Geaster*").

Expanded basidiomes 18–28 mm wide; exoperidium arched, splitting in 10–12 slightly hygroscopic subequal revolute rays; mycelial layer encrusting debris, peeling off in aged specimens; fibrous layer light colored (13B4); pseudoparenchymatous layer brown (7A9), peeling off irregularly; endoperidial body stalked, globose, papery to finely pruinose, grey to brown (7C9), stalk up to 2.5 mm, often laterally compressed; apophysis reduced; peristome delimited, plicate, conic, darker than the surrounding endoperidium; gleba cottony, brown (8L7).

Spores globose, 4.5–7.0 µm wide, verrucose.

Specimen examined— **ARGENTINA. La Rioja**: Sañogasta, 29°18′51.43″S 67°35′37.36″W, on abundant leaf litter of diverse herbaceous plant species, 27.III.2008, leg. L. Papinutti & G. Rolón (BAFC 51945).

Comments—Although the collected material (BAFC 51945) matches the description of Sunhede (1989), some expanded basidiomes exceed the upper limit indicated in his work and in Bates (2004). This is the first record of *G. schmidelii* for La Rioja province.

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Myxomycetes from China 16: *Arcyodes incarnata* and *Licea retiformis*, newly recorded for China

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Abstract — Two new records for China, *Arcyodes incarnata* and *Licea retiformis*, are reported in this paper. Materials collected from Yunnan and Fujian province are described and illustrated by scanning electron micrographs. Those specimens are deposited in the Herbarium of Mycological Institute of Jilin Agricultural University (HMJAU), Changchun, China.

Key words —taxonomy, slime molds, SEM

Introduction

Our examination of previous myxomycete collections has revealed two new records for China. One is in *Arcyodes*, which is currently accepted as a monotypic genus. The other is in *Licea*, which now comprises about 71 species (Kirk et al. 2008, Lado 2001, 2012–14), of which 13 are reported for mainland China (Chou 1937; Li & Li 1989, 1994; Li 2005) and 11 for Taiwan (Liu et al. 2002).

Materials & methods

Fruiting bodies and microscopic structures were examined using light and scanning electron microscopes (Martin & Alexopoulos 1969; Li et al. 1993, Li & Li 1995). Permanent slides were mounted in Hoyer's reagent (Martin & Alexopoulos 1969). Colored slides were prepared according to Robbrecht (1974) by spreading the capillitium in a drop of 94% alcohol, determining color after one minute, and then mounting in Hoyer's reagent. Color terms are given according to the Flora of British Fungi (1969). The specimens are deposited in the Herbarium of the Mycological Institute, Jilin Agricultural University (HMJAU).

Taxonomy

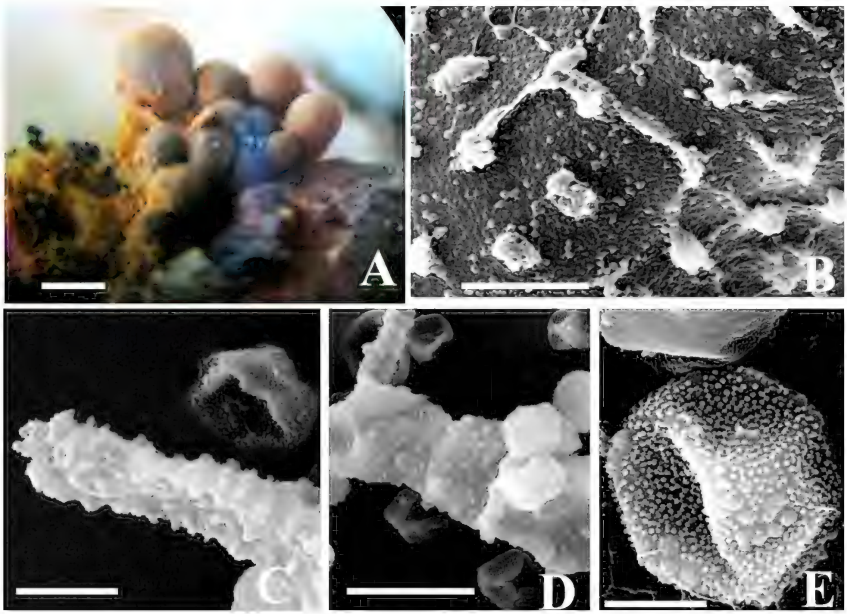

Plate 1. *Arcyodes incarnata*: A, fruiting bodies. B, inner surface of peridium. C, D, part of the capillitium and spores. E, spore. Scale bars: A = 1 mm; B, E = 2μm; C = 5 μm; D = 10 μm.

Arcyodes incarnata (Alb. & Schwein.) O.F. Cook, Science, N.Y. 15: 651, 1902. Pl. 1

Sporocarps aggregated, sessile, crowded and heaped, 0.5–0.7 mm in diam., fawn, fading to dull ochraceous. Hypothallus inconspicuous. Peridium single, membranous, somewhat opalescent, persistent, irregularly dehiscent above, yellow to colourless by transmitted light, inner surface with many prominent warts, low irregular ridges, sometime forming a net with small protuberances. Columella absent. Capillitium tubular, elastic, branched and anastomosed, pale yellow by transmitted light, mostly 4 μm in diam., with numerous inflations, decorated with many cogs and irregular edges, sometimes with a faint reticulation. Spores free, pale pink in mass, yellowish pale to colourless by transmitted light, 7–9.5 μm in diam., globose to subglobose, densely warted, with scattered groups of more prominent warts.

Specimen examined: CHINA, Yunnan Province, Puer, on the bark surface of a dead log, 28 August 2011, Zhang Bo1101 (HMJAU10301).

Comments: *Arcyodes incarnata,* which has been recorded from North America, Europe, and Africa (Martin & Alexopoulos 1969, Lado 1994), is apparently rare in Asia. The Yunnan specimen has slightly larger spores than the type specimen

(6–8 μm in diam.). However, the Yunnan and type specimen are similar in habitat on dead logs, a pale pink spore mass, faintly reticulate capillitium, and pale copper-colored sporocarps.

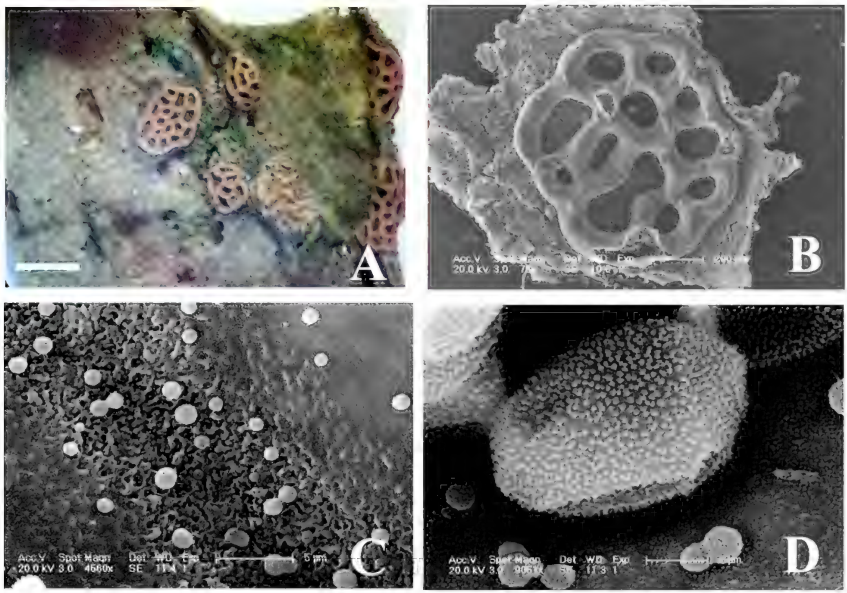

Plate 2. *Licea retiformis*: A, B, fruiting bodies. C, part of peridium. D, spore. Scale bar: A = 1 mm.

Licea retiformis Nawawi, Trans. Br. mycol. Soc. 60(1): 153, 1973. Pl. 2

Fructifications plasmodiocarps, gregarious, reticulation 0.6–3 mm in extension, pale yellowish brown, fading to pale brown. Plasmodiocarps 90–100 μm in diameter, appearing smooth on the surface. Hypothallus inconspicuous. Peridium single, membranous, firm, translucent. Dehiscence by longitudinal slit, decorated with loose small mesh. Columella absent. Capillitium absent. Spores free, pale ochraceous in mass, yellowish pale to colourless by transmitted light, subglobose to ovate, 9–11 × 8.5–12.5 μm, densely warted.

Specimens examined: CHINA, Fujian Province, Fuzhou, on the bark surface of a dead log, 9 July 2010, Li Ming0031 (HMJAU10302); Li Ming0042 (HMJAU10303).

Comments: This is the only *Licea* species with reticulate plasmodiocarps. *Licea retiformis,* which has been reported from Japan (Yamamoto 1998), Malaysia (Nawawi 1973), and Taiwan (Liu el at. 2002), is here newly recorded from mainland China. The two Fujian specimens have slightly shorter spores than the type specimen (9.3–13.5×8.5–12.5 μm, Nawawi 1973).

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Tuber microverrucosum and *T. huizeanum* — two new species from China with reticulate ascospores

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Abstract — Two new species of *Tuber* are described and illustrated. *Tuber microverrucosum* is recognized by its brown verrucose ascomata, blackish gleba, and broadly ellipsoid to subglobose ascospores with a large-meshed regular reticulum. *Tuber huizeanum* is diagnosed by its brown glabrous ascomata, brown gleba, and broadly ellipsoid ascospores with a regular reticulum. Molecular phylogenetic analysis supports the erection of the two new species.

Key words — *Ascomycota*, truffle, taxonomy

Introduction

Truffles are abundant in some regions of China (Garcia-Montero et al. 2010, Wang & Liu 2011, Chen et al. 2011a,b) from which more than 20 species have been described during the 25 years since Liu described *Tuber taiyuanense* B. Liu in 1985 (Liu 1985; Wang 1988; Wang & Li 1991; Wang et al. 1998; Wang & He 2002; Tao et al. 1989; Chen et al. 2005; Chen & Liu 2007). Of these more than half are considered valid species (Cao 2010). That there are likely more new species awaiting discovery in China is confirmed by our ongoing research (Fan et al. 2011, 2012a,b). We describe and illustrate here two additional new species from Yunnan.

Materials & methods

Morphological studies

Truffles were collected from Huize County and a mushroom market in Kunming, Yunnan Province. Macroscopic characters were described from fresh specimens. Microscopic characters were described from razor-blade sections of fresh specimens

Table 1. *Tuber* specimens used in molecular studies

Species name	Voucher specimen	Country of origin	Reference	ITS
T. borchii Vittad.	GB62	Italy	Bonito et al. 2010	HM485342
	GB45	Italy	Bonito et al. 2010	HM485344
T. canaliculatum Gilkey	JT23942	USA	From GenBank (unpublished)	GQ221456
	OSC59072	USA	Bonito et al. 2010	HM485347
T. foetidum Vittad.	ZB-2489	Hungary	Halász et al. 2005	AJ557544
	ZB3454	Finland	Orczán et al. 2010	FN568055
T. gardneri Gilkey	TK1779	USA	From GenBank (unpublished)	AY558808
T. huizeanum	BJTC FAN144	China	This paper	JN870100
	BJTC FAN186	China	This paper	JQ910651
T. linsdalei Gilkey	L63	USA	Bonito et al. 2010	HM485370
T. liui A.S. Xu	HKAS 48269	China	Chen & Liu 2007	DQ898182
T. maculatum Vittad.	FHS-390	Serbia	Marjanović et al. 2010	FM205649
	K(M) 17936	—	Brock et al. 2009	EU784428
T. microverrucosum	BJTC FAN142	China	This paper	JN870099
T. puberulum Berk. & Broome	TL11885	Denmark	Tedersoo et al. 2006	AJ969626
	BI-32	Hungary	Halász et al. 2005	AJ557537
T. separans Gilkey	ZB-32	Hungary	Halász et al. 2005	HM485388
	JT32463	Mexico	From GenBank (unpublished)	GQ221448
T. scruposum R. Hesse	CMI-UNIBO 2192	Armenia	From GenBank (unpublished)	DQ011846
	CMI-UNIBO 2201	Armenia	From GenBank (unpublished)	DQ011845
T. zhongdianense X.Y. He et al.	HKAS 45388B	China	Chen & Liu 2007	DQ898186
	Wang0299	China	Chen & Liu 2007	DQ898187
T. melanosporum Vittad.	A59	France	From GenBank (unpublished)	AF106878
	—	—	Roux et al. 1999	AF132501

mounted in 3% KOH, Melzer's reagent, or 0.1% (w/v) cotton blue in lactic acid. The specimens are deposited in BJTC (Biology Department Herbarium, Capital Normal University). For scanning electron microscopy (SEM), spores were scraped from the dried gleba, placed onto doubled-sided tape, mounted directly on an SEM stub, coated with gold-palladium, and then examined and photographed with a HITACHI S-4800 SEM.

Molecular methods

Herbarium samples were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 ml tube together with one 3 mm diameter tungsten carbide ball. Total genomic DNA was then extracted using the PeqLabE.Z.N.A._Fungal DNA kit following the manufacturer's protocol. The ITS region was amplified with PCR using the primers ITS1/ITS4 (White et al. 1990). PCR was performed in 50 µl reactions containing DNA template 2 µl, primer (10µM) 2 µl each, 2 × Master Mix (Tiangen Biotech (Beijing) Co. Ltd.) 25 µl. PCR reactions were run as follows: an initial denaturation at 95°C for 3 min, followed by 30 cycles at 95°C for 2 min, 55°C for 25 s, 72°C for 2 min, and a final extension at 72°C for 10 min. The PCR products were sent to Invitrogen Biotechnology Co. Ltd. (Beijing, China) for purifying, sequencing, and editing. The other sequence data of ITS rDNA included in this study were downloaded from GenBank. GenBank numbers are shown in Table 1.

Phylogenetic analyses

DNA sequences were aligned with Clustal X (Thompson et al. 1997) and manually adjusted with Se-Al v.2.03a (Rambaut 2000). The aligned dataset was analyzed with maximum parsimony (MP) using PAUP*4.0b10 (Swofford 2002). MP analysis was conducted using heuristic searches with 1000 replicates of random-addition sequence, tree bisection reconnection (TBR) branch swapping algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap (BS) analysis was performed with 1000 replicates, each with 10 random taxon addition sequences. TBR branch swapping was employed. The Bayesian analysis was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001; Ronquist & Huelsenbeck 2003) with two sets of four chains (one cold and three heated) and the stoprule option in effect, halting the analyses at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100, and the first 25% trees were removed as burn-in. Bayesian posterior probabilities (PP) were obtained from the 50% majority rule consensus of the remaining trees. Two sequences derived from *Tuber melanosporum* (AF132501, AF106878) were used as outgroups.

Results

Molecular phylogenetics

343 of 634 characters were found to be parsimony-informative. Maximum parsimony analysis produced one most parsimonious tree (Fig.1) with a length (TL) of 907 steps, consistency index (CI) of 0.7310, retention index (RI) of 0.8501, and rescaled consistency index (RCI) of 0.6214 (for all sites).

ITS sequence-based phylogenies (Fig. 1) group the new *Tuber microverrucosum* in a clade with *T. foetidum* with moderate support (BS = 74, PP = 0.99). The two *T. huizeanum* sequences grouped in a clade with strong support (BS = 99, PP = 1.00) and were sister (but with very low support) to *T. zhongdianense*.

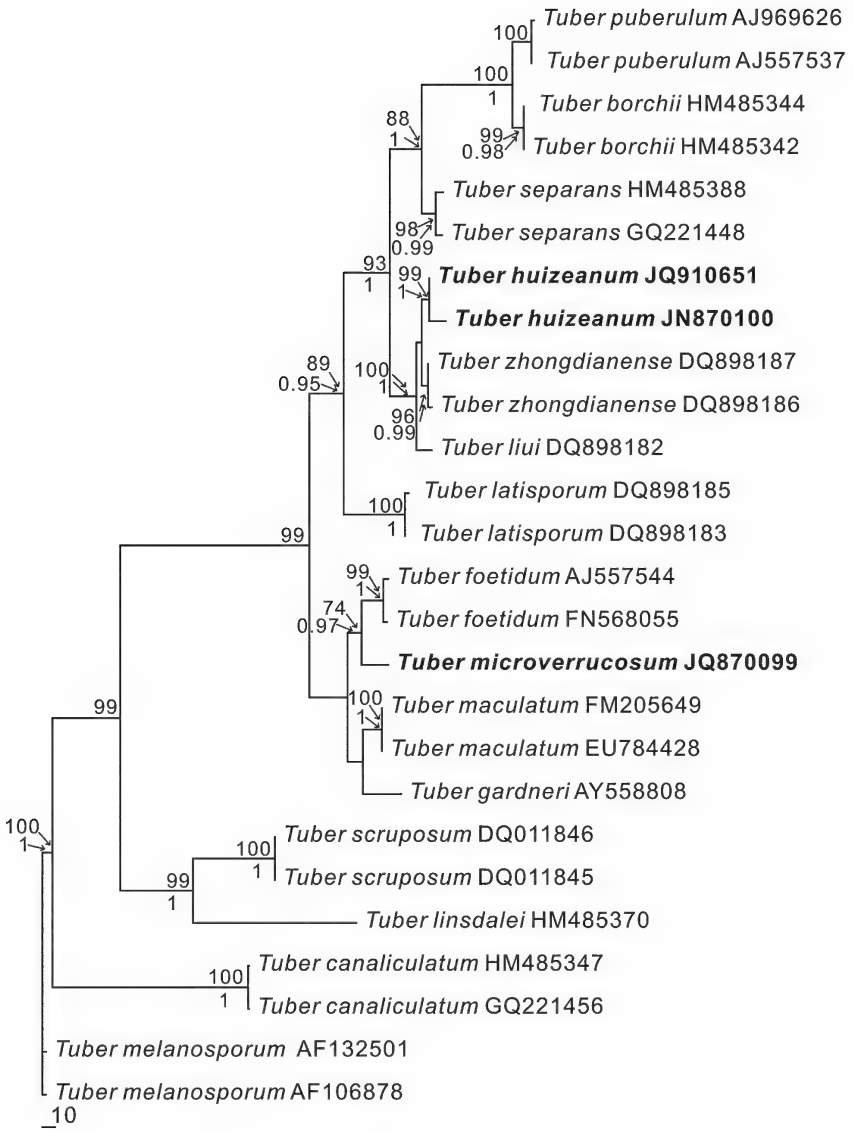

FIG. 1. Phylogeny derived from maximum parsimony analysis of the ITS rDNA sequences of some *Tuber* species with reticulate ornamentations on the ascospore surface, using *T. melanosporum* as outgroups. Bootstrap values of more than 70% (BP ≥ 70) from 1000 replications are shown above the respective branches. Bayesian posterior probabilities (PP) were estimated and clades with PP > 0.95 (95%) are marked under the branches.

Taxonomy

Tuber microverrucosum L. Fan & C.L. Hou, **sp. nov.** FIG. 2 a–d

MYCOBANK MB 563657

Differs from other *Tuber* species with reticulate ascospores by its brown verrucose ascomata, blackish gleba, and broadly ellipsoid ascospores.

TYPE: China. Yunnan Province, Kunming, from the Kunming mushroom market. 20 Jan. 2011, Jin-Zhong Cao 105 (**Holotype**, BJTC FAN142).

ETYMOLOGY: *microverrucosum* (Lat.), referring to the minute verrucose surface of the ascomata.

ASCOMA globose or irregular subglobose, 2.2 cm in diam., light brown to brown when fresh, the surface glabrous and distinctly minute verrucose, the places without verrucose show gray-brown or pale gray. Odor slight when young and middle pungent after maturity. PERIDIUM 250–400 μm thick including the verrucose, two layers; outer layer pseudoparenchymatous, composed of subglobose and angular cells (7.5–)10–20(–25) μm in diam, with slightly thickened walls, yellowish-brown towards the surface; inner layer texture intricate, the hyphae hyaline and thin walled, 5–7.5 μm in diam. GLEBA white at first, then brown to dark brown and blackish at maturity, marbled with larger and rare pale white veins which originate from all of the peridium. ASCI ovate or subglobose, sessile, 75–100 × 50–75 μm, (1–)2–4(–6)-spored. ASCOSPORES broadly ellipsoid to subglobose, a few globose, at first hyaline, becoming brown to dark brown at maturity, thick-walled 2.5–3 μm, surface ornamented with regular reticulum, 4–6(–7.5) μm high, 25–32.5 × 20–27.5 μm, Q = 1.10–1.25, in 2–6-spored asci, and 37.5–42.5 × 27.5–32.5 μm, Q = 1.30–1.36, in 1-spored asci, excluding the ornamentation, the meshes large and variable in size, usually 3–4 meshes across the width.

COMMENTS — *Tuber microverrucosum* is distinguished by its brown ascomata with minutely distinct verrucose surface, blackish gleba, and broad ellipsoid to subglobose ascospores covered with regular deep reticulum.

Two European species, *Tuber foetidum* and *T. scruposum*, are similar to the new species. *Tuber foetidum* differs in having a smooth or indistinctly verrucose peridium, a pale brown gleba and yellow-brown ascospores (Pegler 1993), whereas *T. scruposum* has a distinct verrucose peridium, and narrow elliptical ascospores (Lange 1956).

Several North American species that also have verrucose ascomata and reticulate ascospores include *T. canaliculatum*, *T. linsdalei*, *T. longisporum* Gilkey, and *T. gardneri*. *Tuber canaliculatum* differs in large (>50 μm long) ascospores (Gilkey 1954), while *T. linsdalei* has subglobose ascospores and a reticulum with numerous small meshes ("alveoli small and numerous"; Gilkey

1954). Long ellipsoid ascospores distinguish *Tuber longisporum* and *T. gardneri* from *T. microverrucosum*.

ITS sequence analyses (Fig. 1) support erection of *T. microverrucosum*. *Tuber microverrucosum* and *T. foetidum* grouped in the same clade with moderate support value (BS = 74, PP = 0.99), indicating that they are closely related but morphologically quite distinct.

Tuber huizeanum L. Fan & Yu Li, **sp. nov.** Fig. 2 e–i

MycoBank MB 563658

Differs from other *Tuber* species by its brown glabrous ascomata, brown colored gleba, and very broadly ellipsoid ascospores with a regular reticulum.

Type: China. Yunnan Province, Huize County, in soil under conifers, 28 Dec. 2011, Jin-Zhong Cao 513 (**Holotype**, BJTC FAN186).

Etymology: *huizeanum* (Lat.), referring to the type locality of the new species.

Ascomata 1.5–2.0 cm, subglobose, solid, surface smooth, glabrous, sometimes with a few furrows, white to yellow-white at first, light brown to brown at maturity. Odor slight, not pungent. Peridium 350–400 μm thick, two layers; outer layer 100–150 μm, pseudoparenchymatous, composed of subangular or subglobose cells mostly 10–25(55) μm, with slightly thickened and light yellowish walls, hypha-like hairs absent from the outermost cells; inner layer composed of intricately interwoven hyphae, hyaline, thin-walls, branch, septate, 2.5–5 μm, intermixed with a few of hyphae up to 10 μm in diam. Gleba brown at maturity, marbled with large and rare, branch white veins coming from the peridium. Asci subglobose, ellipsoid or irregular, hyaline, slightly thick-walled, 80–115 × 75–100 μm, sessile, 1–4-spored. Ascospores subglobose to very broad ellipsoid, a few of globose, light brown to yellow-brown at maturity, 27.5–42.5 × 25–40 μm, Q = 1.06–1.15, in 2–4-spored asci, and 50–55 × 42.5–45 μm, Q = 1.10–1.25, in 1-spored asci, excluding ornamentation; ornamentation regularly alveolate reticulum, 3–5 μm high, the meshes generally 4–8(10) across the spore width.

Additional material examined: **China. Yunnan Province**, Kunming, from the Kunming mushroom market. 20 Jan. 2011, Jin-Zhong Cao 107 (BJTC FAN144).

Comments — Three species from China are similar to the new species. *Tuber zhongdianense* has similarly colored ascomata and shaped ascospores as *T. huizeanum*. However, *T. zhongdianense* ascomata are obviously puberulent with two types of hairs and the ascospore reticulum is only 2.5 μm high (He et al. 2004). These two characters clearly distinguish the two species. *Tuber liui* differs by its large (38–71 × 27–40 μm) and ellipsoid to long ellipsoid ascospores (Xu 1999) and a peridium with hypha-like hairs. *Tuber latisporum* is differentiated by its white ascomata, blackish gleba, red brown ascospores, and a peridium covered with hypha-like hairs.

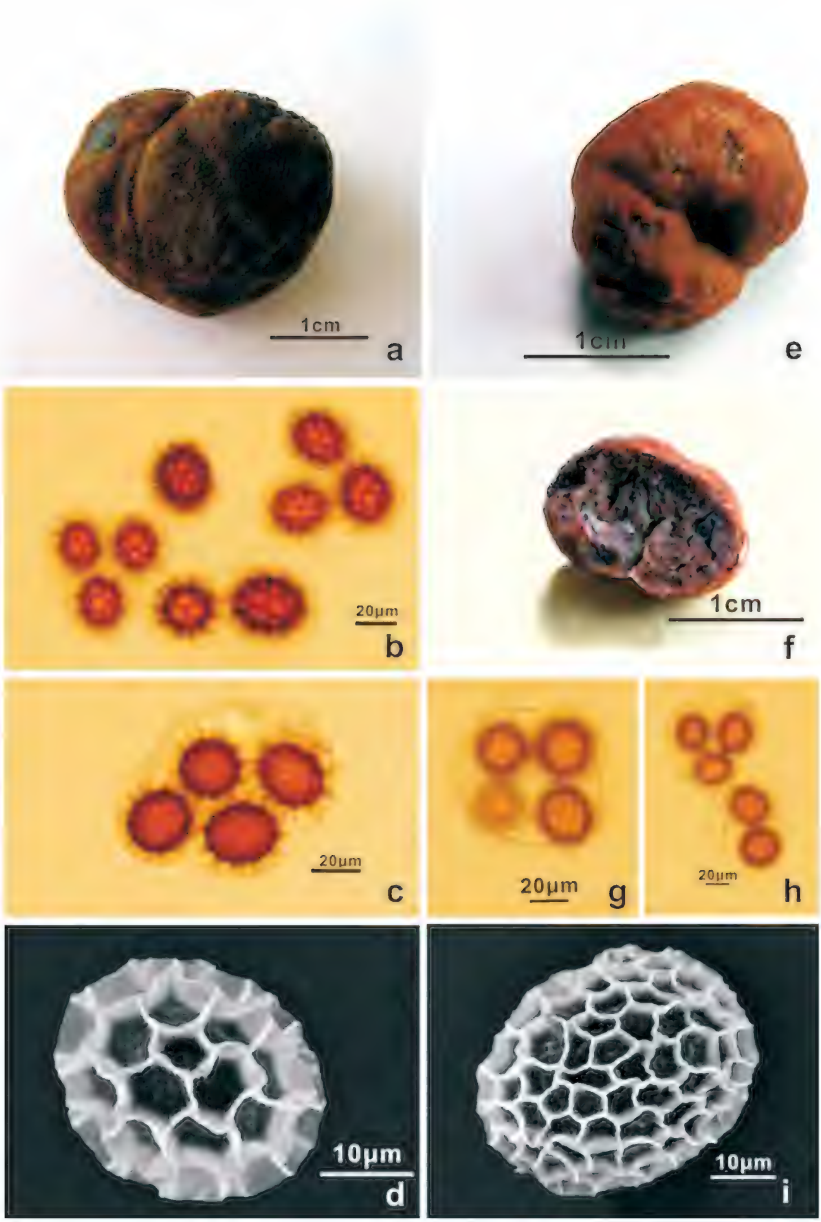

Fig. 2. ***Tuber microverrucosum*** (BJTC FAN142, holotype): a. Ascoma; b–c. Asci and ascospores observed under light microscope; d. Ascospore observed under SEM. ***Tuber huizeanum*** (BJTC FAN186, holotype): e–f. Ascoma; g–h. Asci and ascospores observed under light microscope; i. Ascospore observed under SEM.

The similar European *T. puberulum* can be separated by its ascomata with abundant surface hairs and globose ascospores. *Tuber borchii* has similar ascospores but differs by its dark chocolate brown mature gleba and puberulent peridium. The North American *T. separans* is distinguished by its inconspicuous glebal veins (Gilkey 1954).

Phylogenetic analyses (Fig. 1) support the erection of *T. huizeanum*. The morphologically similar *T. huizeanum* and *T. zhongdianense* form a sister relationship in the phylogeny but with low support (Fig. 1).

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Three new species of *Humicola* from the Qinghai-Tibet Plateau Area, China

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ABSTRACT — Three new species from soil in China, *Humicola chlamydospora, H. tuberculata,* and *H. verruculosa,* are described and illustrated. The type specimens (dried cultures) and living cultures are deposited in the Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP). Isotypes are kept in the Herbarium of Institute of Microbiology, Academia Sinica (HMAS).

KEY WORDS — dematiaceous hyphomycetes, soil fungi, taxonomy

Introduction

During a survey of dematiaceous soil hyphomycetes in China, several unusual species of *Humicola* were collected, of which three are described and illustrated here as new. *Humicola,* a genus established by Traaen (1914), is characterized by possession of micronematous or semi-macronematous conidiophores that are cylindric or slightly inflated. The conidiogenous cells swell apically to form globose to ovoid aleuriospores. Of the 66 taxa listed by Index Fungorum (2012), many are infraspecific, and Seifert et al. (2011) estimate that the genus may contain only 20 valid species.

Cultures were examined after 7 days growth on potato dextrose agar (PDA) (De Bertoldi 1976).

Humicola chlamydospora Y.M. Wu & T.Y. Zhang, **sp. nov.** FIG. 1

MYCOBANK MB 563897

Differs from *Humicola alopallonella* by its multinucleate conidia and from *H. nivea* by its smaller and smooth conidia.

TYPE: China, Tibet: Xigaze, from mountain soil, altitude 2300 m, 14 Sept. 2007, Y.M. Wu (**Holotype** HSAUP II $_{07}$1337; **isotype** HMAS 196265).

ETYMOLOGY: The epithet refers to the chlamydospores of this species.

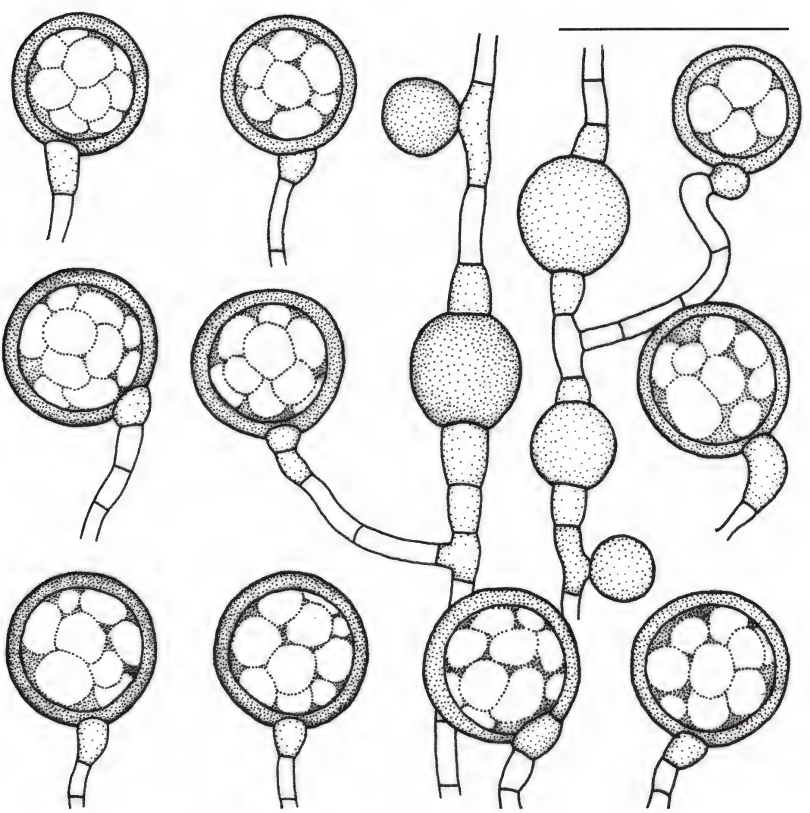

FIG. 1. *Humicola chlamydospora* (ex holotype).
Conidia, conidiophores, conidiogenous cells and intercalary chlamydospores. Scale bars = 25 μm.

COLONIES on PDA at 26°C for 7 days 5–7 cm diam., effuse, velvety, brown to dark brown. Mycelium superficial or immersed; hyphae branched, septate, smooth, subhyaline to light brown, 3–4 μm wide. CONIDIOPHORES subhyaline to light brown, mononematous, micronematous, septate, smooth, 5–7 μm wide. CONIDIA solitary, globose, smooth, golden yellow, thick-walled, (16–)18(–20) μm diam., containing 8–10 oil droplets. Chlamydospores globose, intercalary. Phialospores not seen.

COMMENTS — Morphologically, *H. chlamydospora* resembles *H. alopallonella* Meyers & R.T. Moore (Meyers & Moore 1960) and *H. nivea* De Bert. (De Bertoldi 1976). The new species differs from *H. alopallonella* in having many oil droplets in the conidia and from *H. nivea*, which has rough walled, smaller conidia (8.9–9.4 μm diam.).

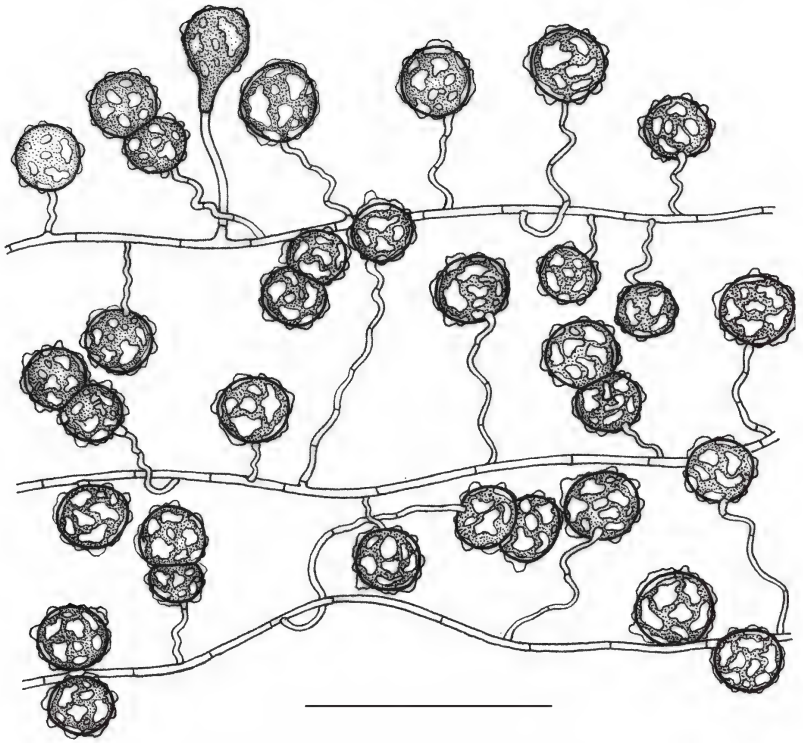

FIG. 2. *Humicola tuberculata* (ex holotype).
Conidia, conidiophores and conidiogenous cells. Scale bars = 25 µm.

Humicola tuberculata Y.M. Wu & T.Y. Zhang, **sp. nov.** FIG. 2

MYCOBANK MB 563888

Differs from *Humicola nivea* and *H. rugosa* by its tuberculate conidia with only a single nucleus.

TYPE: China, Tibet: Shannan, from grassland soil, altitude 3500 m, 9 June 2007, Y.M. Wu (**Holotype** HSAUP II $_{07}$0509; **isotype** HMAS 196266).

ETYMOLOGY: The epithet refers to the tuberculate conidia of this species.

COLONIES on PDA at 26°C for 7 days 4–5 cm diam., effuse, velvety, greyish brown to dark brown. Mycelium superficial or immersed; hyphae branched, septate, smooth, subhyaline to light brown, 1–2 µm wide. CONIDIOPHORES subhyaline, mononematous, micronematous, septate, smooth, 1–2 µm wide. CONIDIA solitary, globose, tuberculate, golden-yellow, thin-walled, (6–)8(–9) µm diam. Phialospores not seen.

COMMENTS — *Humicola tuberculata* resembles *H. nivea* and *H. rugosa* De Bert. (De Bertoldi 1976) in conidial size but differs from these two species in its tuberculate conidia lacking oil droplets.

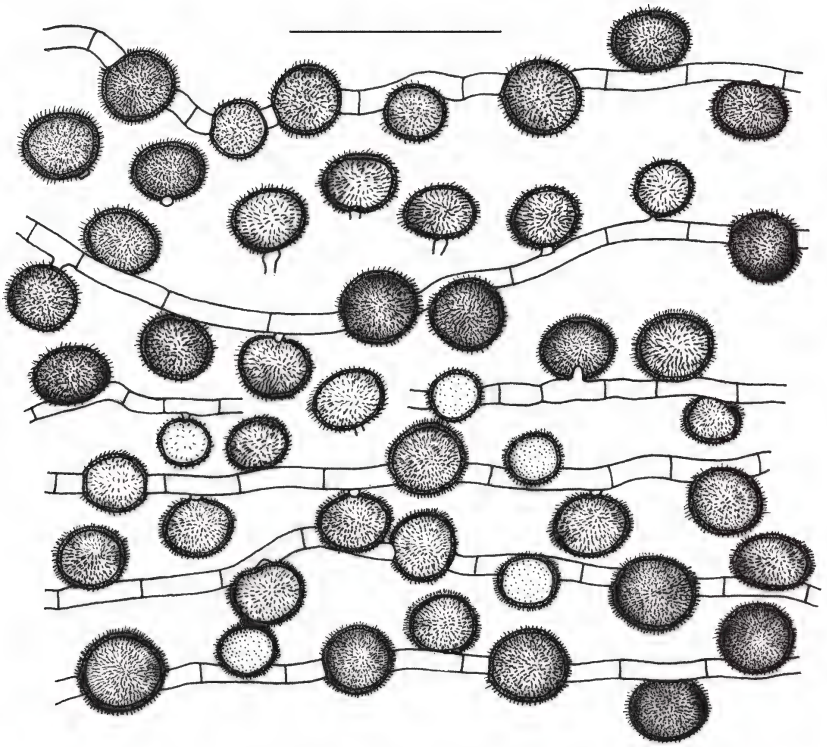

Fig. 3. *Humicola verruculosa* (ex holotype).
Conidia, conidiophores and conidiogenous cells. Scale bars = 25 μm.

Humicola verruculosa Y.M. Wu & T.Y. Zhang, **sp. nov.** Fig. 3

MycoBank MB 563893

Differs from *Humicola dimorphospora* and *H. piriformis* by its larger, ellipsoidal and verrucose conidia.

Type: China, Tibet: Xigaze, from a mountain soil, altitude 2300 m, 14 Sept. 2007, Y.M. Wu (**Holotype** HSAUP II$_{07}$1332; **isotype** HMAS 196264).

Etymology: The epithet refers to the verruculose conidia.

Colonies on PDA at 26°C for 7 days 4–5 cm diam., effuse, velvety, brown to dark brown. Mycelium superficial or immersed: hyphae branched, septate, smooth, subhyaline to light brown, 3–5 μm wide. Conidiophores reduced to conidiogenous cells on side of hyphae, mononematous, micronematous, subhyaline to light brown, smooth, 4–7 μm wide. Conidia solitary, ellipsoidal or ovoid, with a scar at the base, produced singly on the sides of hyphae or on short lateral conidiophores, verruculose, pale yellowish brown to yellowish brown, 8–12 × 5–8 μm. Chlamydospores globose, intercalary. Phialospores not seen.

Comments — Morphologically, *H. verruculosa* is similar to *H. dimorphospora* Roxon & S.C. Jong (Roxon & Jong 1974) and *H. piriformis* De Bert. (De Bertoldi 1976), but its conidia are larger than those of *H. dimorphospora* (3–5 μm diam.) and *H. piriformis* (7–7.2 × 7.1–7.5 μm). Our new species is additionally distinguished from those two species by its ellipsoidal conidia with verrucose conidial wall.

Acknowledgments

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A new species of *Calonectria* causing leaf disease of water lily in China

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Abstract — A species of *Calonectria*, isolated from a leaf spot of water lily (*Nymphaea tetragona*) in Guiyang, Guizhou Province, China was shown to be pathogenic by applying Koch's postulates. Identification based on morphological characters and a comparison of sequences from beta-tubulin and translation elongation factor 1 alpha (tef1) genes supported its status as a new species. *Calonectria nymphaeae* sp. nov. is introduced and compared with similar taxa.

Key words — ITS sequences, taxonomy, hyphomycetes

Introduction

During a survey of hyphomycetes in Guizhou Province, China, various taxa were isolated from different plant hosts. Two strains isolated from a leaf spot of water lily had branched conidiophores and a stipe extension terminating in characteristic vesicles, producing cylindrical, 1–multi-septate conidia; these morphological characteristics are typical of *Cylindrocladium* Morgan (anamorphic *Calonectria* De Not.) (Crous & Wingfield 1994, Crous 2002). In culture the strain also produced a *Calonectria* teleomorph. Recent studies have largely improved our understanding of the phylogeny and systematics among *Calonectria* and allied genera (Lombard et al. 2010a,b,c, Doveri et al. 2010). Lombard et al. (2010c) provided dichotomous and synoptic keys to all *Calonectria* species currently recognized.

The *Calonectria* species isolated from *Nymphaea tetragona* is described as a new species, *C. nymphaeae*. It is illustrated with light micrographs and its uniqueness is confirmed in a phylogram generated from beta-tubulin and translation elongation factor 1 alpha (TEF1) sequence data, which are the regions that provide most valuable insights into relationship between all *Calonectria* species (Schoch et al. 2000, 2001, Crous 2002, Henricot & Culham 2002). Koch's postulates also confirmed that this new taxon was the causal agent of leaf disease of water lily.

Materials & methods

Morphological and cultural studies

Diseased leaves of *Nymphaea tetragona* growing in a pond in Huaxi, Guiyang city, Guizhou Province, China were collected in clean plastic bags and returned to the laboratory. Small tissue pieces (each approximately 5 × 5 mm) were cut from the boundary between healthy and infected parts of leaves, which were previously surface disinfected with 0.5% NaOCl. The pieces were plated onto malt extract agar (2% w/v; MEA; Biolab, Midrand, South Africa) and incubated at 25°C for 7 days under continuous near-UV light. Mycelia grew from the infected samples and were transferred to fresh MEA plates. Cultural characteristics and morphology were determined on MEA, and carnation leaf agar (CLA) [1% water agar (10 g/L) with autoclaved carnation leaves placed onto the medium] (Gams *et al.* 1998) and incubated for 7 days at 25°C under continuous near-UV light, to promote sporulation. A water solution of 60% (v/v) lactic acid without a color dye was used as the mounting medium. Slides were examined under oil immersion with a UB200i microscope (Chongqing UOP Photoelectric Technology, China) at 1000× magnification. Dried cultures of this species were deposited in the Plant Pathology Herbarium of Guizhou University (HGUP). Living cultures were deposited in HGUP and Centraalbureau voor Schimmelcultures (CBS).

DNA sequencing and alignment

DNA was extracted from mycelium using CTAB method. Three loci including fragments of the ITS, β-tubulin (BT), and translation elongation factor 1 alpha (TEF1) gene regions were sequenced. Primers used to sequence these regions were ITS1 and ITS4 (White et al. 1990) for ITS region, T1 (O'Donnell & Cigelnik 1997) and CYLTUB1R (Crous et al. 2004) for the BT region, and EF1-728F (Carbone & Kohn 1999) and EF2 (O'Donnell et al. 1998) for the TEF1 region. Reaction mixtures contained 5 µL of 10 × ThermoPol reaction buffer [200 mM Tris-HCl, pH 8.3, 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$ and 1% Triton X-100], 5 µL of 10 mM $MgSO_4$, 20 ng template genomic DNA, 4 pM of each primer, 4 µL of 2.5 mM dNTPs, 2.5 U of AmpliTaq polymerase, and total volume was adjusted to 50 µL with deionized water. The PCR amplified DNA fragments were fractionated in 1% agarose gels in 0.5 × TBE buffer, and DNA was visualized by ethidium bromide staining and UV illumination. Sequencing was performed with an ABI PRISM 3730 DNA autosequencer using either dRhodamine terminator or Big Dye Terminator chemistry (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California). The DNA sequences of isolates HGUP100003 and

HGUP100004 in ITS, BT and TEF1 regions generated in this study were submitted to GenBank (JF499828, JN984864, JN984866, JN984865, JX546573 and JX546579). The ITS sequences comparison with published sequences was performed using a BLAST search.

Phylogenetic analyses

The combined BT and TEF1 DNA sequence of HGUP100003 was primarily aligned with the ClustalX (Thompson et al. 1997). Alignments files are available in TreeBASE (www.treebase.org/treebase-web/home.html) with study ID 12047. We built up the phylogenetic tree based on combined BT and TEF1 gene regions using the MP (Maximum Parsimony) by PAUP* 4.0b10 (Swofford 2002) and Bayesian methods by MrBayes v3.1.1 (Ronquist & Huelsenbeck 2003). A partition homogeneity test (Farris et al. 1994) was applied to evaluate the feasibility of combining the datasets. In the MP analyses, trees were inferred using a heuristic search option with tree bisection reconnection (TBR) branch swapping and 1000 random sequence additions replicates, maxtrees were 100, branches of zero length were collapsed and all parsimonious trees were saved. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC). Bootstrap analyses (Hillis & Bull 1993) were based on 1000 replications.

The model of evolution in Bayesian analysis was TPM2uf+G estimated by jModelTest 0.0.1 (Posada 2008). A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v3.1.1 for the BT and TEF1 sequence datasets. Two independent runs of four MCMC chains were run simultaneously from random trees for 1,010,000 generations and sampled every 100 generations for the combined analysis of the gene partitions. Both runs converged on the same likelihood score and tree topology, and the first 2500 trees were discarded as the burn-in phase of each analysis. Posterior probabilities were determined from the remaining 7600 trees.

Pathogenicity test

Pathogenicity of this fungus was determined by inoculating healthy leaves of *N. tetragona* with 5 mm diameter mycelial plugs, cut from the margins of 10-day-old actively growing cultures; the control was treatment with sterile agar plugs. Both inoculated and control plants were kept in a moist chamber at 25°C for 4 days, and observed for symptom development. Infected leaves were collected and the fungus was reisolated in MEA medium and compared with the original isolate.

Results

Phylogenetic analysis

Our initial BLAST searches identified DNA sequences of isolates HGUP100003 and HGUP100004 to be most closely related to those of *Calonectria eucalypti*. The alignment sequences indicated the ITS sequence of this fungus was 99.6% identical (only two base pairs difference) to four sequences (GQ280631, GQ280632, GQ280633, GQ280634) of *C. eucalypti* with 499-bp characters. Sequence GQ280633 originated from the *C. eucalypti* ex-

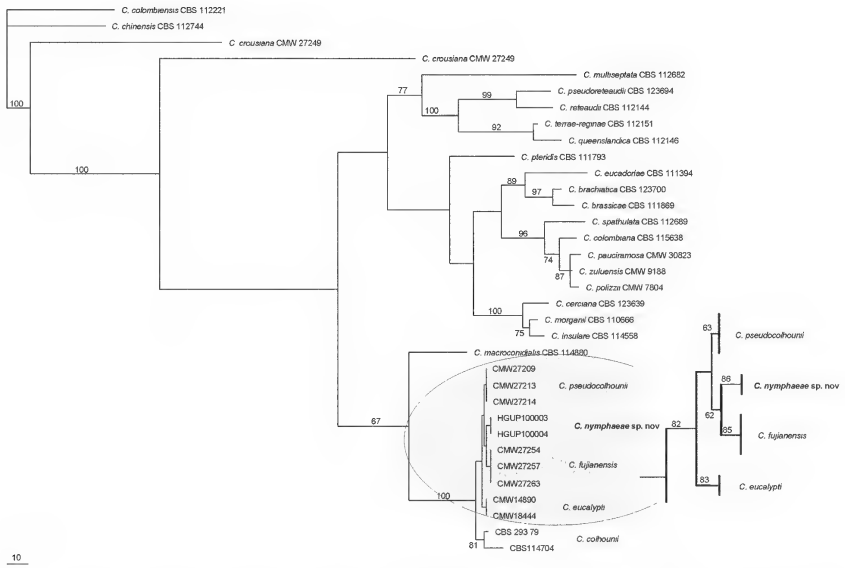

FIG. 1 One parsimonious tree based on combined sequences of BT (beta-tubulin) and *tef1* (translation elongation factor 1 alpha) for *Calonectria nymphaeae* and 24 other *Calonectria* species downloaded from GenBank. *C. colombiensis* (CBS 112221) and *C. chinensis* (CBS 112744) serve as outgroups. The detailed phylogenetic relationships between *C. nymphaeae*, *C. fujianensis*, *C. eucalypti*, and *C. pseudocolhounii* are shown in the amplified part of the bottom right corner. Bootstrap values ≥ 50% are shown above or below branches. Thickened branches indicate Bayesian PP ≥ 95 %.

type strain (CMW18444); therefore we originally identified our two isolates as *C. eucalypti*.

However, *C. eucalypti* is a species complex and our *Calonectria* isolates were revealed as a cryptic species following comparisons of sequences of BT and TEF1 gene regions. Partition homogeneity tests for combinations of the two gene regions used, yielded a P-value of 0.001. Based on the tree topologies and a P-value of 0.001 (Cunningham 1997, Dettman et al. 2003), the two gene regions were combined. The aligned sequence data matrix contained 27 taxa, including 2 outgroups and 1024 characters. Among them, BT has 515 characters, and TEF1 has 509 characters. Of these, 294 were parsimony informative. Ten most parsimonious trees were obtained, one to represent the topology of the strict consensus tree selected for presentation (FIG. 1). The tree was described as follows: Tree Length (TL) = 833, CI = 0.6423, RI = 0.8012, HI = 0.3577, and RC = 0.5146. In this parsimonious tree, 25 species of *Calonectria* resided in a strongly supported large monophyletic clade with a bootstrap value of 100%.

Among them, two isolates of *C. nymphaeae* clustered together with a 86% supported value. *Calonectria nymphaeae*, *C. pseudocolhounii*, *C. eucalypti*, *C. colhounii*, and *C. fujianensis* are related species supported by a high bootstrap value (100%). However, only *C. fujianensis* showed a closer relationship with *C. nymphaeae* with a moderate bootstrap value (62%), and *C. nymphaeae* and *C. eucalypti* are distant.

The topology in Bayesian analysis was nearly identical to that of the MP analyses. The Bayesian trees are therefore not shown, but the statistically supported clades (posterior probabilities ≥ 0.95) are marked with a thickened line in the parsimony tree (Fig. 1).

Taxonomy

Calonectria nymphaeae Yong Wang bis, S.Y. Qin, P. Tan & K.D. Hyde, **sp. nov.**

MycoBank MB 800112 Fig. 2

Differs from *Calonectria pseudocolhounii* and *C. fujianensis* by its slightly larger macroconidia and its longer, narrower ascospores.

Type: China, Guizhou Province, Guiyang, Huaxi, Huaxi Garden, in a pond, from the diseased leaves of *Nymphaea tetragona* Georgi (*Nymphaeaceae*), dried sporulating MEA medium cultures, July 2010, S.Y. Qin (**Holotype**, HGUPd100003; **isotype**, CBS; ex-type living cultures—HGUP100003, CBS 131802; GenBank—JF499828, JN984864, JX546573).

Etymology: in reference to the host from which the fungus was isolated.

Ascomata solitary or in groups, orange to red, becoming red-brown with age, perithecial; in section, apex and body yellow to orange, base red-brown, subglobose to ovoid, 360–450 µm high, 320–380 µm diam. Peridium rough, consisting of 2 thick-walled layers; outer layer of textura globulosa, 30–60 µm wide; becoming more compressed towards inner layer of textura angularis, 13–16 µm wide; becoming thin-walled and hyaline towards the center, outer cells 28–35 × 10–18 µm; inner cells 10–13 × 5–7 µm, ascomata base up to 90–100 µm wide, consisting of dark red, angular cells, merging with an erumpent stroma, cells of the outer wall layer continuing into the pseudoparenchymatous cells of the erumpent stroma. Asci 4-spored, clavate, 100–145 × 12–16 µm, tapering to a long thin stalk. Ascospores aggregated in the upper third of the ascus, hyaline, guttulate, fusoid with rounded ends, straight to slightly curved, 2–3-septate, not or slightly constricted at the septa, 51–71 × 5.5–6.5 µm (av. = 66 × 6.0 µm). Cultures homothallic. Conidiophores with a stipe bearing a penicillate suite of fertile branches, stipe extensions, and terminal vesicles. Stipe septate, hyaline, smooth, 250–300 × 3–4.5 µm; stipe extensions septate, straight to flexuous, 120–240 µm long, 3–5 µm wide at the apical septum, terminating in a clavate vesicle, 3–5 µm diam. Conidiogenous apparatus 50–100 µm long, and 30–70 µm wide; primary branches aseptate or 1-septate, 18–28 × 4–5

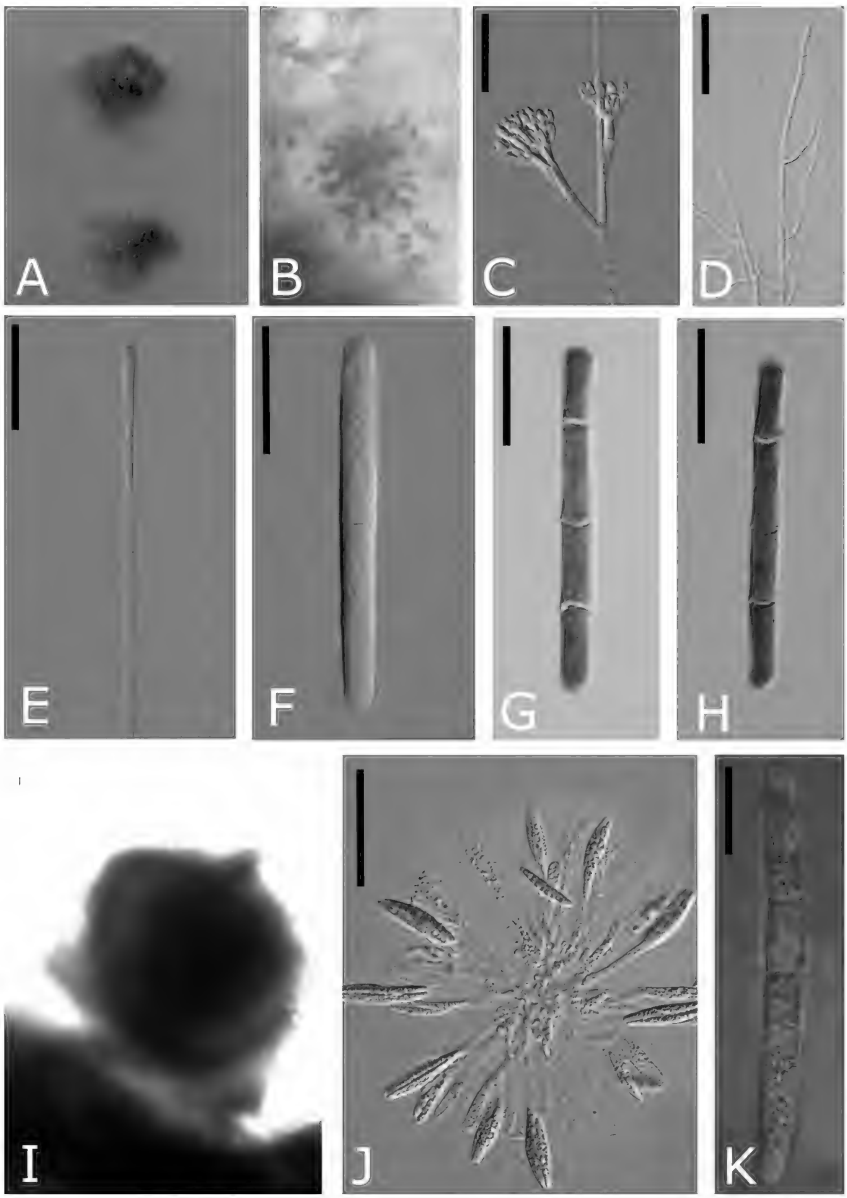

Fig. 2 *Calonectria nymphaeae*: A–B. cultures on MEA; C. macroconidiophore; D. fertile branches; E. clavate vesicle; F. macroconidium; G–H. macroconidia stained by cotton blue; I. perithecium; J. asci; K. ascospore. Scale bars: C–E = 50 μm; F–H = 20 μm; J–I = 100 μm; K = 15 μm.

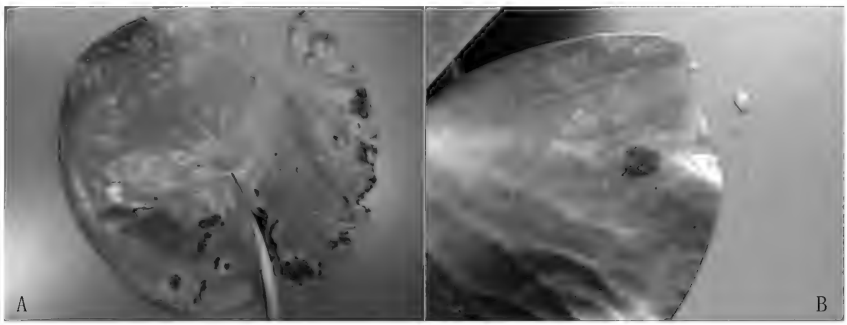

Fig. 3 A: Naturally occurring symptoms on *Nymphaea tetragona* caused by *Calonectria nymphaeae*; B: Symptoms on *N. tetragona* leaves by inoculation.

μm; secondary branches aseptate, 9–15 × 3–4 μm; tertiary branches aseptate, 6–12 × 2.5–3.5 μm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform, hyaline, aseptate, 9–12 × 2.5–3 μm; apex with minute periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, 55–63 × 5.3–6.3 μm (av. = 61 × 5.9 μm), 3–4-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime.

Additional specimen examined: **China, Guizhou Province**, Guiyang, Huaxi, in a pond, from diseased leaves of *Nymphaea tetragona*, August 2011, Y. Wang, (HGUP100004; GenBank—JF984866, JN984865, JX546579).

Comments: *Calonectria nymphaeae* is similar to species in the *C. colhounii* complex (Chen et al. 2011) that all have yellow ascomata, (1–)3-septate ascospores, and clavate vesicles in the anamorph state. *Calonectria nymphaeae* is morphologically most similar to *C. pseudocolhounii* S.F. Chen et al. and *C. fujianensis* S.F. Chen et al. However, the macroconidia of *C. nymphaeae* are slightly larger than those of *C. pseudocolhounii* (av. 60 × 4.5 μm) and *C. fujianensis* (av. 52.5 × 4.5 μm). The ascospores of *C. nymphaeae* are longer but narrower than those of *C. pseudocolhounii* (av. 56 × 6.5 μm) and *C. fujianensis* (av. 55.5 × 6.8 μm). In addition, *C. pseudocolhounii* and *C. fujianensis* were isolated from terrestrial hosts (*Eucalyptus dunnii* and *E. grandis*), while *C. nymphaeae* was isolated from a hydrophyte.

Pathogenicity test

Following mycelial inoculation, *N. tetragona* leaves exhibited dark brown to black necrotic spots (Fig. 3b) after 4 days, which were very similar to those of natural infection (Fig. 3a). *C. nymphaeae* was successfully reisolated from the artificially inoculated leaves of *N. tetragona*, thus establishing proof of pathogenicity. Controls remained healthy and the leaves did not yield any microorganisms at 4 days.

Discussion

The phylogenetic results indicate that ITS sequence data poorly resolves closely related species of *Calonectria*, while BT and TEF1 sequence data are more effective DNA markers for phylogenetic analyses. In our study, morphological comparison indicated that *C. nymphaeae* possessed some unique characters that differ from related species. The phylogenetic analyses based on BT and TEF1 regions confirmed that the two *C. nymphaeae* isolates clustered together as an independent branch with creditable bootstrap (86%) and posterior probabilities values (1.00) (FIG. 1). The clade including *C. nymphaeae*, *C. pseudocolhounii*, *C. eucalypti*, *C. colhounii*, and *C. fujianensis* was supported by high statistical values (BP 100%, PP 1.00), which is consistent with morphological comparison. By combining morphology and phylogeny we conclude that *C. nymphaeae* is a novel species. Koch's postulates showed the taxon to be the causal agent of the leaf spot disease of *N. tetragona*. According to Peerally (1991), *Cylindrocladium hawksworthii* Peerally (anamorphic *Calonectria hawksworthii* L. Lombard et al.) was the pathogen of a common foliage disease on *Nymphaea lotus* in Mauritius. However, *C. nymphaeae* produces conidia with 3–4 septa, but those of *Cylindrocladium hawksworthii* have only 1 septum. The phylogenetic analyses also proved these two pathogens were not the same species.

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Two species of *Agaricus* sect. *Xanthodermatei* from Thailand

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Abstract —*Agaricus murinocephalus* is described and illustrated as a new species. This taxon, presently known only from Thailand, is characterized by a context that generally does not discolor on exposure, the lack of a phenolic odor, a cap covered by very fine, appressed, dark grey squamules outside the grey disc on a whitish background, and a broken collar-like annulus. *Agaricus endoxanthus*, previously reported from South America, Europe, and Asia, is recorded for the first time in Thailand. Morphological and published molecular analyses place both taxa in *A.* sect. *Xanthodermatei* based on.

Key words —*Basidiomycota, Agaricaceae*, new species, taxonomy, tropics

Introduction

Agaricus is a genus that comprises many edible species but also includes a small number of species that are toxic if eaten. These toxic taxa do not generally cause death but may cause gastrointestinal upsets. Most toxic species are members of *A.* sect. *Xanthodermatei* (Parra 2008).

Agaricus sect. *Xanthodermatei* was established by Singer (1948) with the type species *A. xanthodermus* Genev. Recent molecular phylogenetic analyses of samples from Europe and North America showed the section to be monophyletic (Callac & Guinberteau 2005, Challen et al. 2003, Geml et al. 2004, Kerrigan et al. 2005). Inclusion of tropical species in molecular analysis by Zhao et al. (2011) also confirmed monophyly of the group. *Agaricus* sect. *Xanthodermatei* is characterized mainly by its chemical reactions as all section members have a negative Schäffer reaction and bright yellow positive KOH reaction (Parra 2008). Other important features shared by many taxa in sect. *Xanthodermatei* are a transient yellowing discoloration in the pileus surface and stipe base when bruised or cut, phenolic or iodine-like odors, and toxic compounds that can cause gastrointestinal disorders in humans. Surprisingly *A. biannulatus* Mua et al., a recently published new species in this section has neither yellow context discoloration nor a phenolic or iodine-like odor (Parra et al. 2011). Sect. *Arvenses* and sect. *Minores* (also with yellow staining species) can be distinguished from sect. *Xanthodermatei* by their more persistent color change, positive Schäffer reaction, anise seed or almond-like odors, and lack toxic compounds causing gastrointestinal upset when ingested (Parra 2008).

As part of our ongoing studies of macrofungi in northern Thailand (Van de Putte et al. 2010), including *Agaricus* (Zhao et al. 2011) and its relatives (Zhao et al. 2010), we describe here two *A.* sect. *Xanthodermatei* species from the region: one a newly proposed species and the other a first record for Thailand.

Materials & methods

Collections were made in forests and grassy areas in northern Thailand between 2004 and 2010. Specimens were photographed in situ and gathered and wrapped in aluminum foil or kept separately in a box in order to avoid mixing or crushing, and returned to the laboratory for treatment. Odor and color change upon bruising were recorded when collecting. Descriptions of other macrocharacters, chemical tests, and further photography of fresh samples were carried out as soon as possible after return from the field, generally following Largent (1986). Color terminology follows Kornerup & Wanscher (1978). Specimens were dried overnight in a food drier, sealed in plastic bags, and deposited in the BIOTEC Bangkok Herbarium (BBH), with duplicates in the H.D. Thiers Herbarium at San Francisco State University (SFSU). Herbarium acronyms follow Thiers (http://sweetgum.nybg.org/ih continuously updated).

Micromorphological features were documented from examination of dried specimens according to Largent (1986). Particular attention was given to the anatomy of the pileipellis, stipitipellis and partial veil, and features of the hymenophoral trama, basidiospores, basidia and cystidia. Measurements of anatomical features (spores, basidia and cheilocystidia) are presented based on at least 20 measurements, and include x, the mean of length by width ± SD, Q, the quotient of basidiospore length and width, and Qm, the mean of Q-values ± SD. The phylogenetic relationships of the two species included herein are published in Zhao et al. (2011).

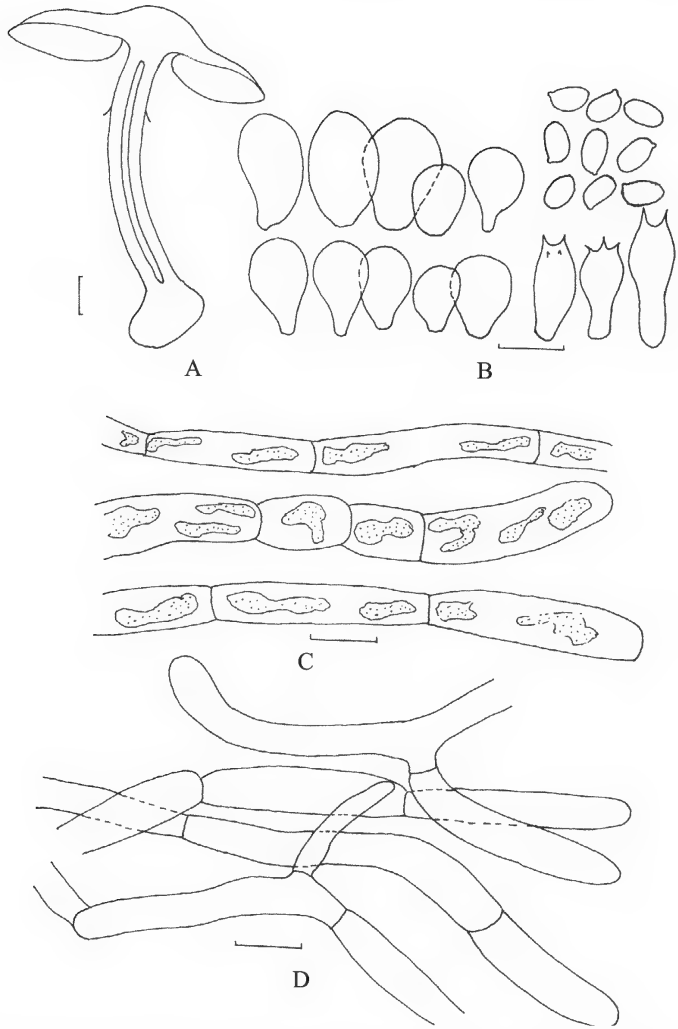

Plate 1. *Agaricus murinocephalus* (Holotype BBH 19560): A. Longitudinal section of basidiocarp; B. Basidiospores, basidia and cheilocystidia; C. Pileipellis hyphae; D. Annulus hyphae. Scale bars: A = 10 mm; B–D = 10 µm.

Taxonomy

Agaricus murinocephalus R.L. Zhao, Desjardin & K.D. Hyde, **sp. nov.** Pl. 1, 3A–E

MycoBank MB564460

Differs from other species of *Agaricus* sect. *Xanthodermatei* by its lack of (or very slight) yellow context discoloration on exposure, lack of phenolic or iodine-like odor, pileus disc with very fine grey squamules on a white background, and broken collar-like annulus.

Type: Thailand. Chiang Mai Prov., Mae Taeng, Ban Mae Sae village, on Hwy 1095 near 50 km marker, 19°14.599′N 98°39.456′E, altitude 962 m, 10 June 2006, coll. Todd Osmundson ZRL3044 (**Holotype**, BBH 19560; **isotype** SFSU; GenBank, JF691555).

Etymology: from *murinus* = mouse-grey, and *cephalus* = headed; referring to the grey-colored pileus.

Pileus 30 mm diam. when young, then expanding to 50–55 mm diam., convex with distinct broad umbo; margin deflexed, splitting with age, never uplifted; surface dry, covered with fibrils or very finely squamulose; disc generally greyish brown (5F5) to black or sometimes with greenish tones; ornamentation disrupted towards the whitish margin and appearing minutely pulverulent on a whitish background. Context 3 mm thick at disc, white. Lamellae free, crowded, with 4 series of lamellulae, 5–6 mm broad, ventricose, at first white, then light orange grey (6B2), later greyish brown (8D3), finally dull brown to dark brown. Stipe 40–70 × 4–5 (apex) × 5–10 (base) mm, cylindrical to subclavate above a bulbous base, hollow; surface smooth, silky, white. Annulus 2 mm broad, collar-like, broken, ephemeral, white. Odor pleasant. Surfaces of stipe and pileus staining reddish-brown on touching; context lacking color change or very slightly discoloring yellow at the base of stipe on cutting.

Macrochemical reactions KOH reaction bright yellow; Schäffer reaction negative.

Basidiospores 5.5–7 × 3–4 µm, [x = 6 ± 0.4 × 3.5 ± 0.3 µm, Q = 1.4–2.0, Q_m = 1.75 ± 0.35, n = 20], elongate-ellipsoid, without germ pore, smooth, brown. Basidia 12–19 × 6–8 µm, hyaline, smooth, 4-spored. Cheilocystidia scattered, 9–24 × 8–13 µm, pyriform to broadly clavate, hyaline, smooth. Pleurocystidia absent. Pileipellis a cutis formed from hyphae 5–10 µm diam., some constricted at septa, cylindrical, walls hyaline, containing brown vacuolar pigment. Annulus composed of hyphae of 4–7 µm diam. that are smooth, hyaline and branched.

Habit solitary or scattered in small groups in open areas of forest.

Additional material examined: **THAILAND**, **Chiang Rai Prov.**, Mae Sai, Doi Tung, 3 August 2006, coll.: Rui-lin Zhao ZRL3092 (BBH 19608, SFSU).

Agaricus endoxanthus Berk. & Broome, J. Linn. Soc. Bot. 11: 548. 1871. Pl. 2, 3f–h

Pileus 65 mm diam., convex to plano-convex; disc with a broad, flattened or slightly depressed umbo; pileus margin exceeding the lamellae as a white edge; surface dry, covered by brownish-grey (8E2) to grey (8E5) fibrils, radially fissured exposing the underlying white context that turns pink in wet conditions; at disc fibrils darker and aggregated into small, scattered fibrillose squamules. Context firm, white. Lamellae free, crowded, with 10 or more series of lamellulae, up to 4 mm broad, straight or ventricose, greyish red to dull red (8C3) when young, brown to dark brown (8F4) with age; edges paler than the sides. Stipe 60 × 4–12 mm, cylindrical below, with a slightly tapered

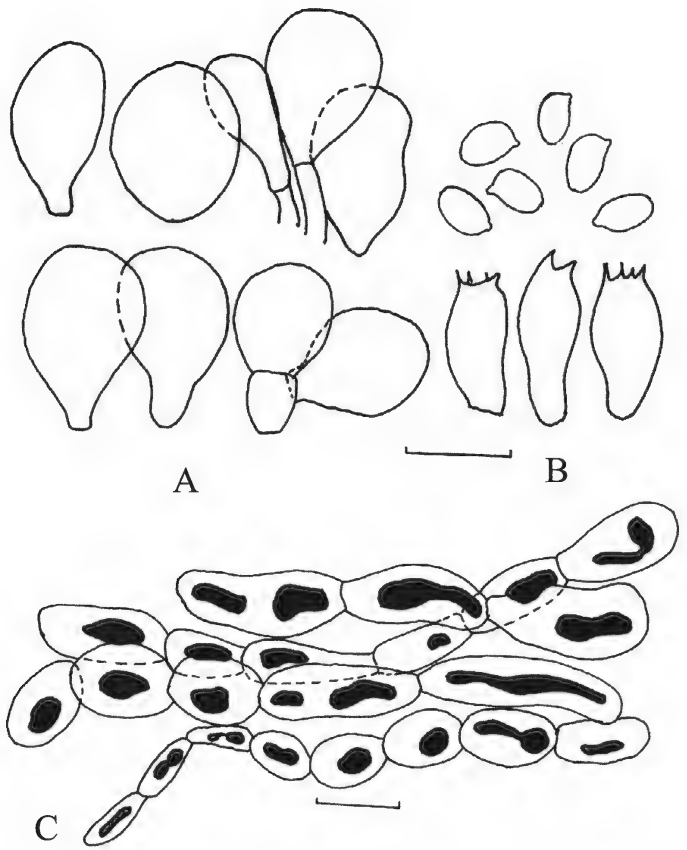

PLATE 2. *Agaricus endoxanthus* (BBH 19611): A. Cheilocystidia; B. Basidiospores and basidia; C. Pileipellis hyphae. Scale bars: A–B = 10 µm; C = 25 µm.

apex: base slightly swallowed with white rhizomorphs, hollow; surface both above and below the annulus glabrous, brownish grey (8E2). ANNULUS superior, persistent, pendent, single, up to 11 mm broad; lower surface slightly floccose; edge entire, white, and sometimes with brown granules. ODOR indistinct, not phenolic or almond-like. Base of stipe and centre of disc strongly staining yellow on cutting, without color change on touching.

MACROCHEMICAL REACTIONS KOH reaction yellow; Schäffer reaction negative.

SPORES 5–6 × 3–4 µm [x = 5.4 ± 0.6 × 3.5 ± 0.5 µm, Q = 1.25–2, Q_m = 1.55 ± 0.45, n = 20], ellipsoid with attenuate apex in most cases, without germ pore, smooth, brown. BASIDIA 12–17 × 5–7 µm, clavate, 4-spored. CHEILOCYSTIDIA (9–)11–17 × 8–12 µm, rarely seen in old specimens, mostly pyriform, some turbinate

or subsphaerical, hyaline, smooth. Pleurocystidia absent. Pileipellis a cutis, composed of cells variable in size and shape, many 25–44 × 17–25 µm, others 17–25 × 5–7.5 µm, distinctly constricted at the septa, with dark brown vacuolar pigments. Annulus composed of hyphae of 5–7.5 µm diam., which are hyaline, smooth and curved. Stipitipellis with hyphae similar to those of the pileipellis, but slightly smaller.

Habit solitary in soil, in open areas of forest.

Material examined: **THAILAND**, **Chiang Mai Prov.**, Mae Taeng Dist., Ban Pha Deng Village, 19°17.123′N 98°44.009′E, elev. 900 m., 13 August 2006, coll.: Rui-lin Zhao ZRL3095 (BBH 19611, SFSU; see also Zhao et al. 2011).

Discussion

Although some morphological characters of *A. murinocephalus,* such as the slightly reddish-brown discoloration on bruising of the pileus and the lack of typical phenolic odor are not typical for *A.* sect. *Xanthodermatei*, the molecular phylogenetic position (Zhao et al. 2011), negative Schäffer reaction, positive KOH reaction, and pileipellis hyphae with vacuolar pigments support this new species in this section. Furthermore, the previous molecular work separates this new species from *A. endoxanthus*, *A. microvolvatulus* Heinem., *A. xanthodermulus* Callac & Guinb., *A. xanthodermus*, and *A. xanthosarcus* Heinem. & Gooss.-Font. Specimen NTF61 (see sp. 39 in the Zhao et al. 2011 phylotree) represents another possible species sister to *A. murinocephalus* that our molecular analyses show as a different new species. A complete morphological and molecular characterization of specimen NTF61 will be presented in another paper.

The context in *A. murinocephalus* either does not stain yellow or discolors only slightly on exposure. Most sect. *Xanthodermatei* species stain distinctly yellow after being cut, especially at the stipe base of the stipe, e.g. *A. bulbillosus* Heinem. & Gooss.-Font., *A. endoxanthus*, *A. iodosmus* Heinem., *A. moelleri* Wasser, *A. menieri* Bon, *A. pseudopratensis* (Bohus) Wasser, *A. xanthodermus*, *A. xanthosarcus* (Cappelli 1984, Heinemann 1956a, 1978; Kerrigan 1986, Nauta 2001). In some species, such as *A. phaeolepidotus* (F.H. Møller) F.H. Møller, *A. californicus* Peck, or *A. hondensis* Murrill, a weak yellow or rubescent discoloration occurs on cutting, however all taxa have a phenolic odor and lack a dark greyish pileus (Cappelli 1984, Kerrigan 1986, Nauta 2001) that characterize *A. murinocephalus*. *Agaricus biannulatus*, a recently published species (Parra et al. 2011) in this section also lacks a yellow context discoloration and phenolic or iodine-like odor, but differs from *A. murinocephalus* in a two-layered annulus consisting of two distinct separate limbs. *Agaricus volvatulus* Heinem. & Gooss.-Font. and *A. placomyces* Peck are morphologically similar to our new species in that both have blackish pilei and a bulbous stipe. *Agaricus volvatulus*, however, has a distinct phenolic odor and its pileus is darkly pigmented overall,

Plate 3. ***Agaricus murinocephalus***: A–C. Holotype BBH 19560; D–E. BBH 19608. ***Agaricus endoxanthus*** (BBH 19611): F. Basidiomata; G. Cheilocystidia; H. Pileipellis hyphae.

while *A. placomyces* has an elastic annulus that may remain attached to the pileus margin even after pileus expansion (Heinemann 1978, 1980; Kerrigan et al. 2005).

Agaricus endoxanthus is another species with dark appressed squamules on the pileus, a large membranous-floccose annulus, small spores and cheilocystidia, and vacuolar pigments in the pileipellis hyphae (Pegler 1977, 1986; Heinemann 1980). An ITS1+2 sequence of material from Thailand (sample ZRL3095, GenBank JF691554; Zhao et al. 2011) is nearly identical to that of *A. endoxanthus* (GenBank DQ182511) reported in Kerrigan et al. (2005). The fact that only two heteromorphisms separate the two sequences helped us select the appropriate epithet for the Thai material.

Agaricus endoxanthus is similar to *A. pseudoniger* Heinem. & Gooss.-Font. from Congo and *Agaricus rotalis* K.R. Peterson et al. from Hawaii (Peterson et al. 2000). *Agaricus pseudoniger* differs in having a brownish-black pileus and larger cheilocystidia (18–30 × 9.5–18.5 µm; Heinemann 1980, 1956b). *Agaricus rotalis* is morphologically nearly indistinguishable from *A. endoxanthus* (cf. comparison in Kerrigan et al. 2005), but they differ by three heteromorphisms in their ITS1+2 sequences.

Acknowledgments

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Hymenochaete in China. 4. *H. parmastoi* sp. nov. and *H. ustulata* new to China

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Abstract — *Hymenochaete parmastoi* is described as new to science. It is similar to *H. minor* and *H. rhabarbarina*, but morphological and molecular (ITS rDNA sequences) evidence show that they are distinct from each other. *Hymenochaete ustulata*, which was collected from Guangdong Province, southern China, is newly recorded in China. It is characterized by anatomical structure (in sect. *Paragymnochaete* and with abundant crystals). Illustrated descriptions of these two species are provided.

Key words — *Hymenochaetaceae*, phylogeny, taxonomy, wood-inhabiting fungi

Introduction

The poroid wood-inhabiting fungi in China have been intensively studied during the last decade (Yuan & Dai 2008a,b; Dai 2010, 2011, 2012; Cui et al. 2011; Dai & Cui 2011; Zhou & Dai 2012), but the corticioid fungi are still poorly studied. As a contribution to the studies of the corticioid fungi in China, a project dealing with the taxonomy and phylogeny of the genus *Hymenochaete* Lév. (Léveillé 1846) is in progress and several intensive surveys in south and southwest China have been carried out since 2010. Among the 1000 specimens collected, several new species and Chinese new records have been found (He & Li 2011a,b, 2012a,b, He & Dai 2012).

More recently, careful morphological study and molecular analysis of some *Hymenochaete* specimens from several provinces and autonomous regions in temperate and subtropical areas of China indicate that they represent an undescribed species, which we describe here as *H. parmastoi*.

One additional specimen collected from Guangdong Province is identified as *H. ustulata*, a rare species new to Chinese fungal flora. This is the first report outside its type locality. Illustrated descriptions of these two species are provided in this paper.

Materials & methods

Morphological studies: Voucher specimens are deposited in the herbarium of Beijing Forestry University (BJFC), and the microscopic procedure follows Dai (2010). In presenting the size range of spores and setae, 5% of the measurements were excluded from each end of the range, and the measurements were given in parentheses. In the text the following abbreviations are used: L = mean spore length (arithmetical average of all spores), W = mean spore width (arithmetical average of all spores), Q = variation in the L/W ratios between the specimens studied (quotient of the mean spore length and the mean spore width of each specimen), n = the number of spores measured from given number of specimens. IKI stands for Melzer's reagent, KOH for 5% potassium hydroxide, and CB is the abbreviation of Cotton Blue in lactic acid. IKI– = inamyloid and nondextrinoid, CB– = acyanophilous. Special color terms follow Petersen (1996).

DNA extraction and sequencing: The F-130 Phire® Plant Direct PCR Kit (Finnzymes, Finland) was employed for DNA extraction and PCR amplification from herbarium specimens according to the manufacturer's instructions. Approximately 700 base pairs of the ITS rDNA were amplified with primers ITS5 (GGA AGT AAA AGT CGT AAC AAG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC; White et al. 1990), using the following procedure: initial denaturation at 98°C for 5 min, followed by 39 cycles of 98°C for 5 s, 58°C for 5 s and 72°C for 5 s, and a final extension of 72°C for 10 min. DNA sequencing was performed at Beijing Genomics Institute, and all new sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov).

Phylogenetic analysis: The phylogeny was inferred from the ITS sequences of 51 *Hymenochaete* species. *Pseudochaete corrugata* (Fr.) S.H. He & Y.C. Dai and *P. tabacina* (Sowerby) T. Wagner & M. Fisch. were selected as outgroups. The sequences were aligned using the ClustalX 1.83 (Chenna et al. 2003). Alignments were optimized manually in BioEdit 7.0.5.3 (Hall 1999). Maximum parsimony analysis were performed using PAUP* 4.0b10 (Swofford 2002). Gaps in the alignments were treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm, with all characters given equal weight. Branch supports for all parsimony analysis were estimated by performing 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. The tree length (TL), consistency indices (CI), retention indices (RI), rescaled consistency indices (RC) and homoplasy index (HI) were calculated for each tree generated. Trees were figured in Treeview 1.6.6 (Page 1996).

Taxonomy

Hymenochaete parmastoi S.H. He & Hai J. Li, **sp. nov.** Figs 1–2

MycoBank MB 800487

Differs from other *Hymenochaete* species by its yellowish brown basidiocarps, encrusted setae, and ellipsoid to ovoid basidiospores.

Type: China. Xizang Autonomous Region, Bomi County, on fallen angiosperm trunk, 20.IX.2010, He 367 (**holotype**, BJFC; GenBank, JQ780061).

FIG. 1. Basidiocarps of *Hymenochaete parmastoi* (holotype).

ETYMOLOGY: honoring Erast Parmasto, 1928–2012, eminent Estonian mycologist and author of many significant papers on *Hymenochaete*.

FRUITBODY: Basidiocarps annual, effused, closely adnate, coriaceous, first as small round or irregular colonies, later confluent up to 15 cm or more in longest dimension, 50–250 μm thick in section. Hymenophore smooth, cinnamon to clay-buff, not cracked or with numerous deep crevices with age; margin thinning out, distinct, whitish to yellowish when juvenile, becoming indistinct, concolorous with hymenophore surface when mature.

HYPHAL STRUCTURE: Hyphal system monomitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

SUBICULUM: Tomentum and cortex absent. Hyphal layer locally present, thin. Generative hyphae hyaline to yellowish brown, thin- to thick-walled with a wide lumen, septate, usually branched at a right angle, loosely to densely interwoven, 2–4 μm in diam.

STRATIFIED HYMENIUM: Hyphae in this layer similar to those in subiculum, yellowish brown, thick-walled, more or less agglutinated, interwoven, 2–3 μm in diam. Setal layer composed of 1–3 rows of overlapping setae. Setae not numerous, scattered, subulate, reddish brown, with acute tips, projecting up to 30 μm above the hymenium, (30–)40–75(–95) × (5–)6–10 μm, usually enmeshed in thick hyphal sheath, this usually slightly or heavily encrusted with yellowish or brownish amorphous matters in the tip part. Cystidia and hyphidia absent, but hyphal ends sometimes present in the hymenium, some encrusted

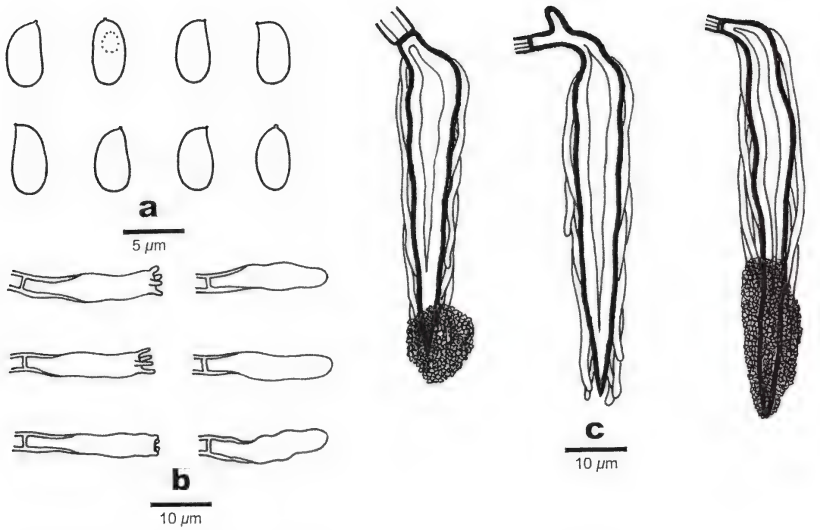

Fig. 2. Microscopic structures of *Hymenochaete parmastoi* (drawn from the holotype). a: Basidiospores. b: Basidia and basidioles. c: Setae.

with amorphous matters. Basidia clavate, with four sterigmata and a simple septum at base, walls thickening at basal part, 15–20 × 3–4.8 μm; basidioles in shape similar to basidia, but slightly smaller.

Spores: Basidiospores ellipsoid to ovoid, hyaline, thin-walled, smooth, IKI–, CB–, (3.8–)4–5.5(–6) × (2.5–)2.6–3.2(–3.5) μm, L = 4.84 μm, W = 2.94 μm, Q = 1.55–1.76 (n = 210/7).

Additional specimens examined: **CHINA. Anhui Prov.**, Huangshan County, Yellow Mountain National Park, on fallen twig of *Rhododendron*, 20.X.2010, He 431 (BJFC); **Guangxi Autonomous Region**, Xing'an County, Mao'ershan Nat. Res., on fallen angiosperm twig, 19.VIII.2011, He 867 (BJFC; GenBank, JQ780063); **Guizhou Prov.**, Leishan County, Leigongshan Nat. Res., on fallen angiosperm twig, 24.VIII.2010, He 266 (BJFC), He 277 (BJFC; GenBank, JQ780064); **Xizang Autonomous Region**, Linzhi County, Lulang, on fallen angiosperm twig, 24.IX.2010, He 388 (BJFC; GenBank, JQ780062), He 391 (BJFC); Gadinggou, on fallen angiosperm twig, 25.IX.2010, He 402 (BJFC); **Yunnan Prov.**, Zhanyi County, Zhujiangyuan Forest Park, on dead angiosperm tree, 11.VI.2011, He 725 (BJFC); Chuxiong, Zixishan Forest Park, on fallen angiosperm trunk, 11.VI.2011, He 741 (BJFC).

Hymenochaete ustulata G.A. Escobar ex J.C. Léger, Cryptog. Mycol. 11: 309, 1990 Figs 3–4

Fruitbody: Basidiocarps annual, effused, closely adnate, coriaceous, first as small colonies, later confluent up to 5 cm or more in longest dimension, 100–150

FIG. 3. Basidiocarps of *Hymenochaete ustulata* (He 104).

μm thick in section. Hymenophore smooth or with scattered small tubercles, vinaceous brown to reddish brown when fresh, becoming pale mouse-gray to vinaceous gray after dry, usually not cracked; margin thinning out, indistinct, paler or concolorous with hymenophore surface.

HYPHAL STRUCTURE: Hyphal system monomitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

SUBICULUM: Tomentum and hyphal layer absent. Cortex well developed, composed of strongly agglutinated hyphae, 20–70 μm thick.

STRATIFIED HYMENIUM: Generative hyphae yellowish brown, thick-walled, agglutinated, 2–3.5 μm in diam. Setal layer thickening, composed of several rows of overlapping setae. Agglomerates of crystals frequently present in the setal layer and subhymenium, 10–30(–50) μm in diam. Setae numerous, subulate, reddish brown, with acute tips, some enmeshed with a thin hyphal sheath, projecting up to 40 μm above the hymenium, (30–)45–70(–75) × 5–9 μm. Cystidia and hyphidia absent. Basidia clavate, with four sterigmata and a simple septum at base, walls thickening at basal part, 15–18 × 3–4 μm; basidioles in shape similar to basidia, but smaller.

SPORES: Basidiospores ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, 4–5 × 2.2–2.8 μm, L = 4.42 μm, W = 2.49 μm, Q = 1.78 (n = 30/1).

SPECIMEN EXAMINED: **CHINA. GUANGDONG PROV.**, Shixing County, Chebaling Nat. Res., on rotten angiosperm trunk, 24.VI.2010, He 104 (BJFC; GenBank, JQ780066).

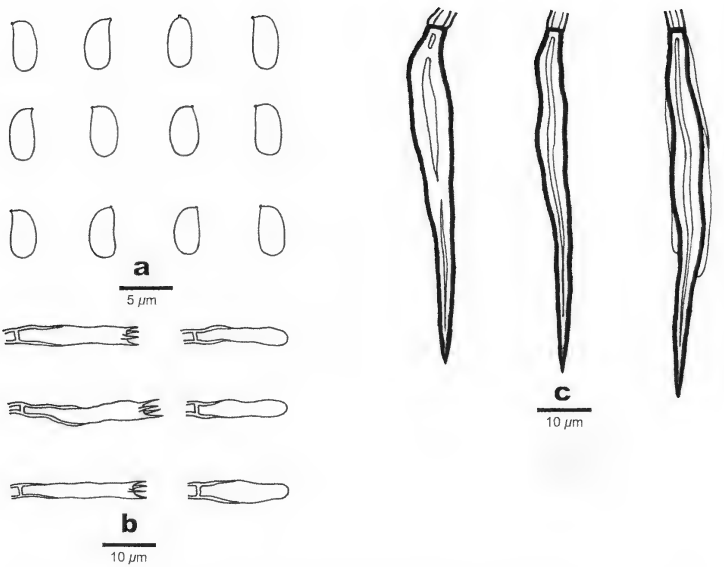

Fig. 4. Microscopic structures of *Hymenochaete ustulata* (drawn from He 104). a: Basidiospores. b: Basidia and basidioles. c: Setae.

Phylogenetic results

Seven ITS sequences were newly obtained in this study (GenBank accession numbers JQ780061–JQ780067). The alignment of the ITS sequences of 53 taxa resulted in 786 sites with 398 parsimony informative characters. One strict consensus tree was yielded from the analysis with TL = 2056, CI = 0.428, RI = 0.645, RC = 0.276 and HI = 0.572 (Fig. 5). The strict consensus tree placed *Hymenochaete* taxa well apart from the outgroups, with *H. parmastoi* strongly supported (bootstrap value = 100%) as a separate lineage and confirming *H. parmastoi* as phylogenetically distinct from any sampled species. The tree also showed *H. parmastoi* closely related to *H. minor* S.H. He & Y.C. Dai and *H. ustulata* closely related to *H. rhabarbarina* (Berk.) Cooke.

Discussion

Hymenochaete parmastoi is characterized by yellowish brown basidiocarps, encrusted setae, and ellipsoid to ovoid basidiospores. In both morphological and phylogenetic aspects, *H. parmastoi* seems very close to *H. minor* (with a 100% bootstrap value), which also has setae encrusted with amorphous matter. However, *H. minor* differs from *H. parmastoi* by its shorter setae (25–50 μm) and smaller basidiospores (3–4.5 × 1.7–2 μm; He & Dai 2012). Moreover, *H. parmastoi* grows mostly in temperate areas, while *H. minor* seems to be a tropical species.

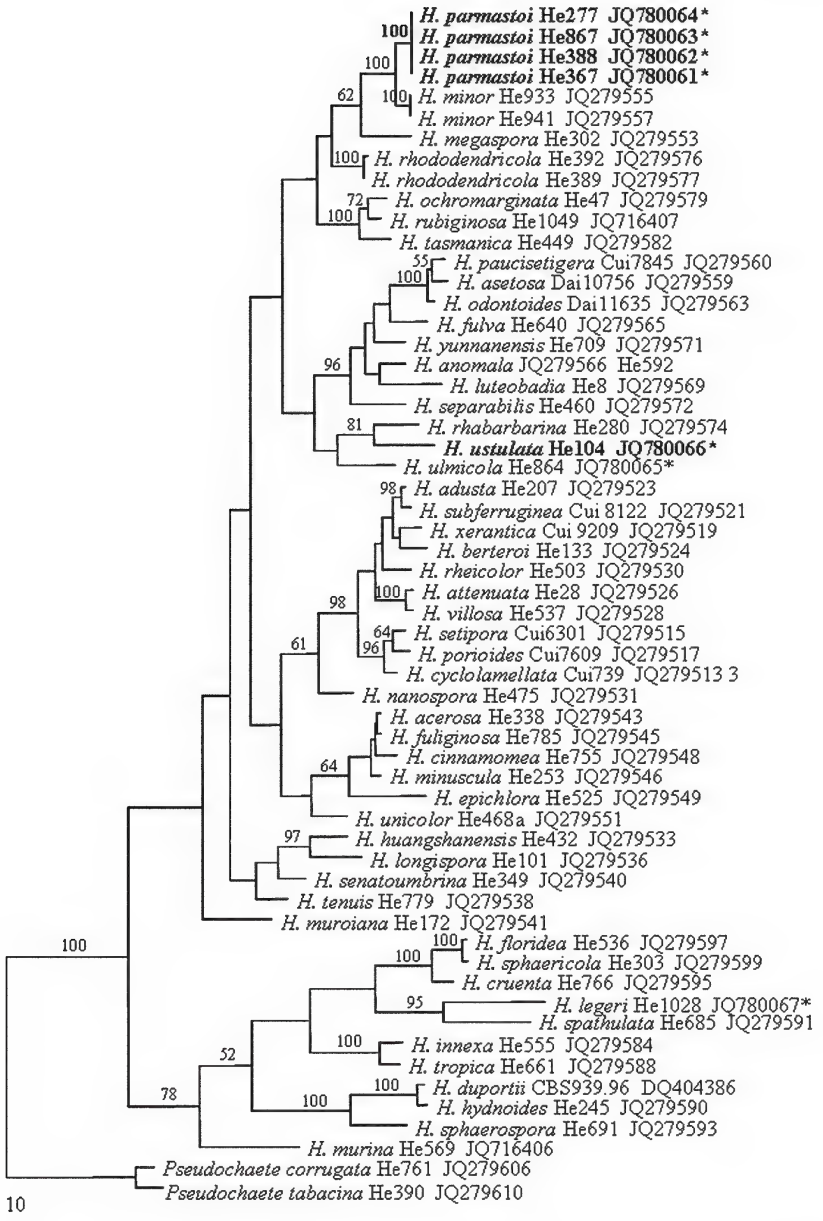
H. parmastoi He277 JQ780064*
H. parmastoi He867 JQ780063*
H. parmastoi He388 JQ780062*
H. parmastoi He367 JQ780061*
H. minor He933 JQ279555
H. minor He941 JQ279557
H. megaspora He302 JQ279553
H. rhododendricola He392 JQ279576
H. rhododendricola He389 JQ279577
H. ochromarginata He47 JQ279579
H. rubiginosa He1049 JQ716407
H. tasmanica He449 JQ279582
H. pauciseligera Cui7845 JQ279560
H. asetosa Dai10756 JQ279559
H. odontoides Dai11635 JQ279563
H. fulva He640 JQ279565
H. yunnanensis He709 JQ279571
H. anomala JQ279566 He592
H. luteobadia He8 JQ279569
H. separabilis He460 JQ279572
H. rhabarbarina He280 JQ279574
H. ustulata He104 JQ780066*
H. ulmicola He864 JQ780065*
H. adusta He207 JQ279523
H. subferruginea Cui 8122 JQ279521
H. xerantica Cui 9209 JQ279519
H. berteroi He133 JQ279524
H. rheicolor He503 JQ279530
H. attenuata He28 JQ279526
H. villosa He537 JQ279528
H. setipora Cui6301 JQ279515
H. porioides Cui7609 JQ279517
H. cyclolamellata Cui739 JQ279513 3
H. nanospora He475 JQ279531
H. acerosa He338 JQ279543
H. fuliginosa He785 JQ279545
H. cinnamomea He755 JQ279548
H. minuscula He253 JQ279546
H. epichlora He525 JQ279549
H. unicolor He468a JQ279551
H. huangshanensis He432 JQ279533
H. longispora He101 JQ279536
H. senatoumbrina He349 JQ279540
H. tenuis He779 JQ279538
H. muroiana He172 JQ279541
H. floridea He536 JQ279597
H. sphaericola He303 JQ279599
H. cruenta He766 JQ279595
H. legeri He1028 JQ780067*
H. spathulata He685 JQ279591
H. innexa He555 JQ279584
H. tropica He661 JQ279588
H. duportii CBS939.96 DQ404386
H. hydnoides He245 JQ279590
H. sphaerospora He691 JQ279593
H. murina He569 JQ716406
Pseudochaete corrugata He761 JQ279606
Pseudochaete tabacina He390 JQ279610
10

FIG. 5. Strict consensus tree obtained from Maximum Parsimony analysis of ITS sequences of *Hymenochaete*. *Pseudochaete corrugata* and *P. tabacina* were used as outgroups. Newly generated sequences are those with *. Parsimony bootstrap values (>50%) are shown.

Two other species, *H. rhabarbarina* and *H. rhododendricola* S.H. He & Hai J. Li, also have setae encrusted with amorphous matter. However, *H. rhabarbarina* differs from *H. parmastoi* by its well-developed hyphal layer, encrusted hyphae, and longer setae (60–100 μm; Parmasto 2001), while *H. rhododendricola* has longer setae (80–130 μm) and larger basidiospores (6–7 × 4–5 μm; He & Li 2011a) than *H. parmastoi*. These two morphologically close allies are distantly related to the *H. parmastoi* clade in the phylogram.

Hymenochaete ustulata was previously reported only in Brazil. The species is diagnosed by its anatomical structure, which contains a well-developed cortex without a hyphal layer (sect. *Paragymnochaete*) and an abundance of crystal agglomerates. The similar *H. separata* G. Cunn. can be separated by its smaller setae (35–45 × 6–7 μm) and larger basidiospores (5–6.5 × 3–3.5 μm; Escobar 1978; Parmasto & Gilbertson 2005). *Hymenochaete dissimilis* G. Cunn. shares similar setae, basidiospores, and agglomerated crystals but differs from *H. ustulata* in its thicker basidiocarps (≤750 μm) and lack of cortex (Cunningham 1957; Léger 1998).

He & Dai (2012) conducted a taxonomic and phylogenetic study of *Hymenochaete* and allied genera in China in which they combined five species of *Hydnochaete* Bres. and *Cyclomyces* Kunze ex Fr. in *Hymenochaete* and transferred five species of *Hymenochaete* were transferred to *Pseudochaete* T. Wagner & M. Fisch. (nom. illegit., non *Pseudochaete* West & G.S. West [*Chlorophyta*]). Including the two species reported in this paper, a total of 55 *Hymenochaete* species have been recorded in China (He & Dai 2012; He & Li 2012b).

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Phylogenetic systematics of the *Gigasporales*

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Abstract — The classification, phylogeny, and evolutionary pathways of the *Gigasporales* are re-evaluated based on concomitant morphological and molecular phylogenetic analyses. Only *Cetraspora* was not supported in the morphology-based tree, while *Quatunica* formed a monophyletic group with its sister genus *Dentiscutata*. Only a few taxa were not completely supported in the SSU rDNA phylogenetic analyses, namely *Dentiscutata* and *Fuscutata* (*Dentiscutataceae*) and *Racocetra* and *Cetraspora* (*Racocetraceae*). However, all trees generated by the LSU, SSU (rDNA), and β-tubulin genes supported the existence of the families with strong support for all genera represented in the LSU rDNA and β-tubulin analyses. In conclusion, the current classification of the *Gigasporales* has a strong morphological and molecular congruency.

Key words — *Glomeromycetes*, gigasporoid, scutellosporoid

Introduction

The arbuscular mycorrhizal (AM) fungi form a symbiotic association with plants, being the most important mutualism between plants and fungi in the nature. These fungi are grouped in the phylum *Glomeromycota*, in which the order *Gigasporales* (Oehl et al. 2011a) with 54 currently described species (Table 1) forms a clade of outstanding interest and particularity for taxonomic, phylogenetic, and ecological reasons. Fungi in this order were grouped according to their spore formation mode on sporogenous cells and phylogenetic analyses of the rDNA and β-tubulin genes (Oehl et al. 2011a). Besides, members of *Gigasporales* do not form vesicles in the colonized roots but rather form auxiliary cells in mycorrhizospheric soils. Recently, Dotzler et al. (2006) reported ~400 million year-old fossil scutellosporoid spores with very structures similar to those in *Gigasporales* (bulbous bases, germinal walls, and

TABLE 1. Species in *Gigasporales**

Dentiscutataceae F.A. Souza et al.
- *Dentiscutata biornata* (Spain et al.) Sieverd. et al.
- *D. cerradensis* (Spain & J. Miranda) Sieverd. et al.
- *D. colliculosa* B.T. Goto & Oehl
- *D. hawaiiensis* (Koske & Gemma) Sieverd. et al.
- *D. heterogama* (T.H. Nicolson & Gerd.) Sieverd. et al.
- *D. nigerita* Khade
- *D. nigra* (J.F. Redhead) Sieverd. et al.
- *D. reticulata* (Koske et al.) Sieverd. et al.
- *D. scutata* (C. Walker & Dieder.) Sieverd. et al.
- *Fuscutata aurea* Oehl et al.
- *F. heterogama* Oehl et al.
- *F. rubra* (Stürmer & J.B. Morton) Oehl et al.
- *F. savannicola* (R.A. Herrera & Ferrer) Oehl et al.
- *F. trirubiginopa* (X.L. Pan & G. Yun Zhang) Oehl et al.
- *Quatunica erythropus* (Koske & C. Walker) F.A. Souza et al.

Gigasporaceae J.B. Morton & Benny
- *Gigaspora albida* N.C. Schenck & G.S. Sm.
- *G. alboaurantiaca* W.N. Chou
- *G. candida* Bhattacharjee et al.
- *G. decipiens* I.R. Hall & L.K Abbott
- *G. gigantea* (T.H. Nicolson & Gerd.) Gerd. & Trappe
- *G. margarita* W.N. Becker & I.R. Hall
- *G. ramisporophora* Spain et al.
- *G. rosea* T.H. Nicolson & N.C. Schenck

Intraornatosporaceae B.T. Goto & Oehl
- *Intraornatospora intraornata* (B.T. Goto & Oehl) B.T. Goto et al.
- *Paradentiscutata bahiana* Oehl et al.
- *P. maritima* B.T. Goto et al.

Racocetraceae Oehl et al.
- *Cetraspora armeniaca* (Błaszk.) Oehl et al.
- *C. gilmorei* (Trappe & Gerd.) Oehl et al.
- *C. helvetica* Oehl et al.
- *C. nodosa* (Błaszk.) Oehl et al.
- *C. pellucida* (T.H. Nicolson & N.C. Schenck) Oehl et al.
- *C. striata* (Cuenca & R.A. Herrera) Oehl et al.
- *Racocetra alborosea* (Ferrer & R.A. Herrera) Oehl et al.
- *R. beninensis* Oehl et al.
- *R. castanea* (C.Walker) Oehl et al.
- *R. coralloidea* (Trappe et al.) Oehl et al.
- *R. fulgida* (Koske & C. Walker) Oehl et al.
- *R. gregaria* (N.C. Schenck & T.H. Nicolson) Oehl et al.
- *R. minuta* (Ferrer & R.A. Herrera) Oehl et al.
- *R. persica* (Koske & C. Walker) Oehl et al.
- *R. tropicana* Oehl et al.
- *R. undulata* T.C. Lin & C.H. Yen
- *R. verrucosa* (Koske & C. Walker) Oehl et al.
- *R. weresubiae* (Koske & C. Walker) Oehl et al.

Scutellosporaceae Sieverd. et al.
- *Orbispora pernambucana* (Oehl et al.) Oehl et al.
- *O. projecturata* (Kramad. & C. Walker) Oehl et al.
- *Scutellospora arenicola* Koske & Halvorson
- *S. auriglobа* (I.R. Hall) C. Walker & F.E. Sanders
- *S. calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders
- *S. crenulata* R.A. Herrera et al.
- *S. dipapillosa* (C. Walker & Koske) C. Walker & F.E. Sanders
- *S. dipurpurescens* J.B. Morton & Koske
- *S. spinosissima* C. Walker & Cuenca
- *S. tricalypta* (R.A. Herrera & Ferrer) C. Walker & F.E. Sanders

*The nomenclature and its authorities are based on Goto et al. (2009, 2010, 2011, 2012), Khade (2010), Lin & Yen (2011), Mello et al. (2012), Oehl et al. (2008, 2010, 2011b,d), Silva et al. (2008), and Tchabi et al. (2009).

Placement of *S. spinossisima* is tentative because its germ shield morphology and phylogeny are not yet sufficiently resolved and need re-analysis.

germination shields). This discovery indicates diversification of *Gigasporales* earlier than suggested by other researchers (Phipps & Taylor 1996, Redecker et al. 2000).

Until 2008 little attention was given to *Gigasporales* classification, although some problems regarding the monophyly and position of *Gigaspora* and *Scutellospora* had been discussed earlier (Kramadibrata et al. 2000, Souza et al. 2005, Silva et al. 2006). Studying the morphological phylogeny of AM fungi, Morton (1990) reported *Gigaspora* as basal to the former *Scutellospora*, and his results were supported by ontogenetic studies (Bentivenga & Morton 1995, Morton 1995, Franke & Morton 1994) and fatty acid profiles (Bentivenga &

Morton 1996). On the other hand, molecular phylogenetic analyses increasingly demonstrated that *Gigaspora* might be monophyletic with *Scutellospora* basal and polyphyletic (Simon et al. 1993, Souza et al. 2005, Redecker & Raab 2006).

Based on concomitant morphological and molecular phylogenetic analyses, Oehl et al. (2008) confirmed the former *Scutellospora* as polyphyletic and proposed four families (*Dentiscutataceae*, *Gigasporaceae*, *Racocetraceae, Scutellosporaceae*) and seven genera (*Cetraspora*, *Dentiscutata*, *Fuscutata*, *Gigaspora*, *Quatunica*, *Racocetra, Scutellospora*) within the sporogenous cell forming AM fungi, i.e. the *Gigasporales*. Countering this revision of *Scutellospora*, Morton & Msiska (2010) reported that such segregation destabilized the taxonomy of the group, proposing that beside the former *Gigaspora* and *Scutellospora*, only one genus (*Racocetra*) described by Oehl et al. (2008) was valid. However, Krüger et al. (2012) provided support for some more genera described by these authors, and a several recent studies with a broader database (e.g. Goto et al. 2010, 2011, 2012, Oehl et al. 2010, 2011b) sustain the revision by Oehl et al. (2008).

Currently, *Gigasporales* comprises five families (*Dentiscutataceae*, *Gigasporaceae*, *Intraornatosporaceae*, *Racocetraceae, Scutellosporaceae*) and ten genera (*Cetraspora*, *Dentiscutata*, *Fuscutata*, *Gigaspora*, *Intraornatospora*, *Orbispora*, *Paradentiscutata*, *Quatunica*, *Racocetra, Scutellospora*) (Oehl et al. 2008, 2011b, Goto et al. 2012). We wish here to evaluate the classification, phylogeny, and evolutionary pathways in *Gigasporales*.

Materials & methods

Morphological phylogenetic analyses

As in Morton & Msiska (2010), we selected just 27 species from *Gigasporales* and 23 characters to construct a matrix for the morphological phylogenetic analysis. In general, characters and the plesiomorphic and apomorphic character states were defined and coded as in Morton & Msiska (2010). We did not include the *Intraornatosporaceae* or *Intraornatospora*, *Orbispora,* and *Paradentiscutata* in our morphological analysis, because these taxa were described after the Morton & Msiska (2010) study.

The phylogenetic analysis and tree construction were performed using Phylogenetic Analysis Using Parsimony (PAUP 4.0b10) (Swofford 2003). The matrix-generated data were calculated by maximum parsimony (MP). *Acaulospora mellea* and *A. laevis* served as outgroups.

Evolutionary phylogenetic terms used in this study (from Wiley et al. 1991) include (1) homoplastic = a character shared by two taxa that does not meet the criteria of homology (i.e., the character does not derive from a common ancestor), (2) plesiomorphic = ancestral character state and apomorphic = derived character state, (3) synapomorphy = a derived character state shared by a group of species with evidence of a common ancestor, (4) symplesiomorphy = an ancestral character state shared by a group of species but useless for phylogenetic analyses since clearly representing common ancestry for all the fungi being analyzed.

Molecular phylogenetic analyses

Partial sequences of rRNA (SSU and LSU) and β-tubulin genes were analyzed independently to reconstruct the phylogeny of the *Gigasporales*. Only β-tubulin exon regions were analyzed, with introns excluded.

Sequences (all obtained from the National Center for Biotechnology Information–NCBI) were aligned with ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999) to obtain a final alignment.

Prior to phylogenetic analysis, the nucleotide substitution model was estimated using Topali 2.5 (Milne et al. 2004). Bayesian (two runs over 1×10^6 generations with a burnin value of 2500) and maximum likelihood (1000 bootstrap) analyses were executed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from Topali 2.5. Neighbor-joining (established with the same model used to construct the Bayesian tree) and maximum parsimony analysis were performed using PAUP*4b10 (Swofford 2003) with 1000 bootstrap replications. *Pacispora scintillans* sequences were used as outgroup for the SSU and LSU rRNA analyses, and *Acaulospora mellea* was outgroup for β-tubulin analysis.

Results

Molecular phylogenetic analysis

All LSU, SSU (rDNA), and β-tubulin gene trees support the families described in the *Gigasporales* (Figs 1–3). The LSU rDNA sequence-based phylogenies support all ten genera (Fig. 1), and the β-tubulin tree supports all genera with species included in the analysis (Fig. 2). Only the SSU rDNA phylogenetic tree did not fully support a few taxa in the *Dentiscutataceae* and *Racocetraceae* (Fig. 3) with no separation shown between *Dentiscutata* and *Fuscutata* or *Racocetra* and *Cetraspora.*

Morphological phylogenetic analyses

The morphology-based phylogeny supported *Gigasporaceae* and *Dentiscutataceae* (Fig. 4). However, *Scutellosporaceae* and *Racocetraceae* grouped together in a monophyletic clade. Our morphological analyses, which included only the same data and taxa used by Morton & Msiska (2010), did excluded taxa described since 2010: *Orbispora*, *Intraornatospora, Paradentiscutata*, and *Intraornatosporaceae*. Of all genera considered, only *Cetraspora* was not supported. *Fuscutata*, *Gigaspora*, *Racocetra*, and *Scutellospora* were monophyletic, and *Quatunica* formed a monophyletic group with *Dentiscutata* (Fig. 4).

Discussion

All LSU, SSU (rDNA) and β-tubulin phylotrees supported the existence of the gigasporalean families with strong support for all genera shown by the LSU rDNA and β-tubulin analyses. As the LSU region is believed to give the best resolution for *Gigasporales* (Souza et al. 2005, Oehl et al. 2008, 2011b),

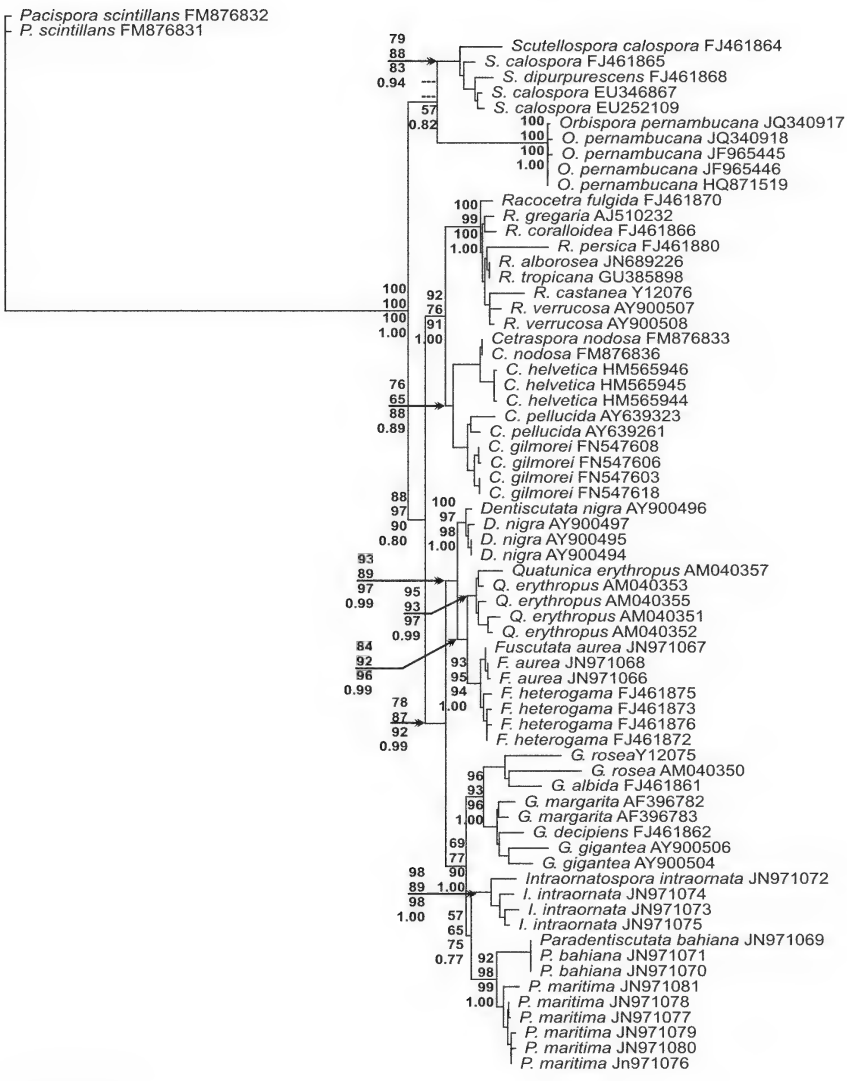

FIG. 1. Phylogenetic reconstruction of the *Gigasporales* obtained from partial LSU rDNA sequences. The NJ, ML, and Bayesian analyses were performed with GTR+G nucleotide substitution model. Sequences are labeled with database accession numbers. Support values are (from up to down) from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. Only topologies with ≥50% bootstrap values are shown. (Consistency Index = 0.55; Retention Index = 0.84).

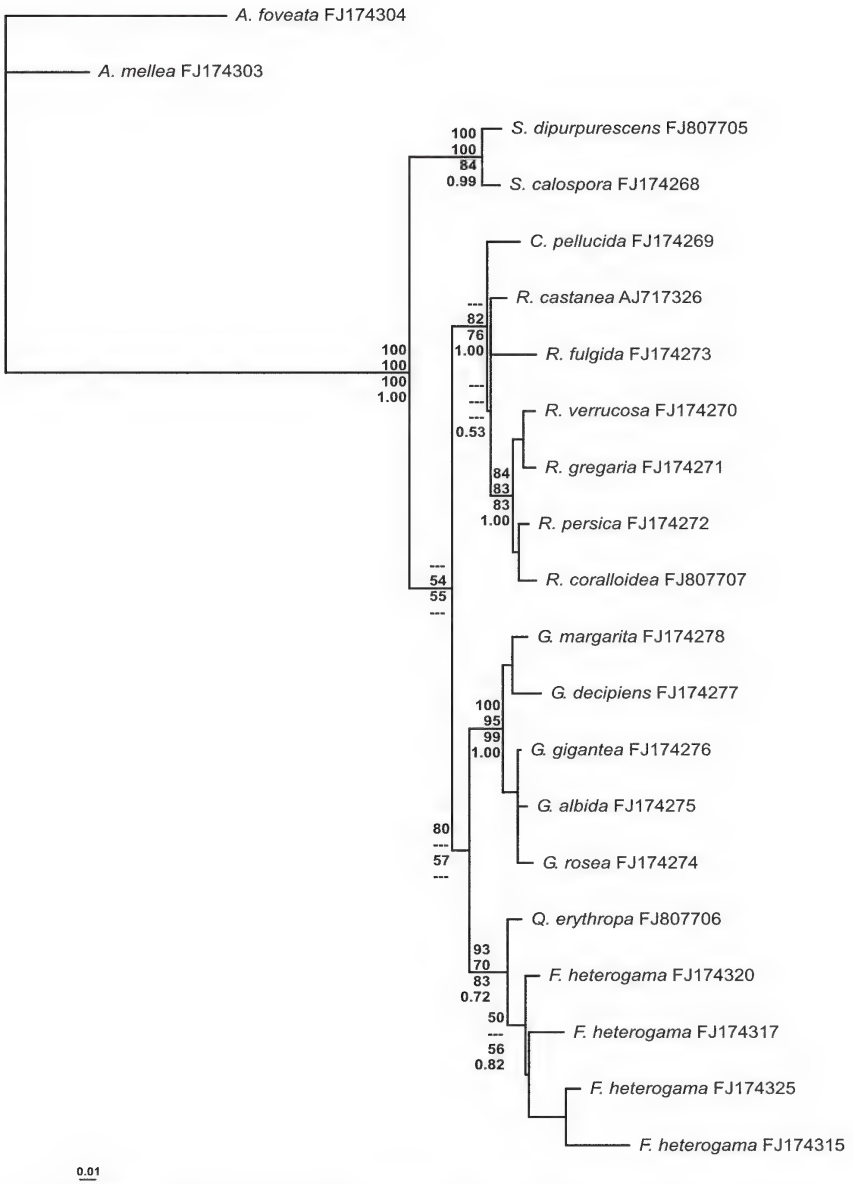

FIG. 2. Phylogenetic reconstruction of the *Gigasporales* obtained from partial β-tubulin sequences. The NJ, ML, and Bayesian analyses were performed with GTR+G+I nucleotide substitution model. Sequences are labeled with database accession numbers. Support values are (from up to down) from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. Only topologies with ≥50% bootstrap values are shown. (Consistency Index = 0.63; Retention Index = 0.68).

FIG. 3. Phylogenetic reconstruction of the *Gigasporales* obtained from partial SSU rDNA sequences. The NJ, ML, and Bayesian analyses were performed with GTR+G+I nucleotide substitution model. Sequences are labeled with database accession numbers. Support values are (from up to down) from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. Only topologies with ≥50% bootstrap values are shown. (Consistency Index = 0.68; Retention Index = 0.74).

we conclude that the current classification by Oehl et al. (2011c) and Goto et al. (2012) has a strong morphological and molecular congruency and support. The SSU rDNA phylogenetic analysis did not completely support only a few taxa — *Dentiscutata* and *Fuscutata* (*Dentiscutataceae*) and *Racocetra* and *Cetraspora* (*Racocetraceae*), which confirms the analyses of Oehl et al. (2008) using a similar database. In the morphology-based tree, only *Cetraspora* was not supported while *Quatunica* formed a monophyletic group with its sister genus *Dentiscutata*. We argue that this might correspond to the fact that *Cetraspora* lacks any unique morphological character and shares (for example) the number of spore walls with several other genera as well as the germination shield color and structure with related genera. Thus far, *Quatunica* has only one important character (spore wall numbers) that separates it morphologically from *Dentiscutata* species. The fact that *Quatunica* is monospecific gives it a low analytical weight.

Of the 23 morphological characters analyzed by Morton & Msiska (2010), we regard eight (1, 2, 3, 4, 6, 8, 11, 12) as synapomorphies that are identical for all gigasporalean species, thereby leaving just 15 characters to divide clades in the order. Among these, Morton & Msiska (2010) report variation in character 21 (germination shield color) between isolates from the same species, which could prevent using this character in phylogenetic analyses, due to its instability. However, we argue that these authors might have included immature spores with germination shields that were not completely differentiated or very old spores in their observations. They might also have considered similar spores from more than one species that represent different phylogenetic clades (e.g. *Cetraspora pellucida*, *Fuscutata savannicola,* and *Dentiscutata scutata*). We also believe that although species show a typical germination shield pattern, natural exceptions may occur in some individuals, due to several reasons such as a malformation.

Morton & Msiska's (2010) characters 9 (spore size), 10 (spore color), and 13 (outer layer surface of the gigasporoid spore wall) do not pass a homology test, because there is no congruence with other characters among the taxa (de Pinna 1991). Moreover, characters 9 and 10 are quantitative, and the most obvious character filter for cladistic analysis rejects continuous or quantitative attributes. Bentivenga et al. (1997) reported significant variation in the average of spore size and color in a single *Glomus clarum* isolate after generations of selection pressure for some phenotypic characters. Bever & Morton (1999) also observed average variation in *Cetraspora pellucida* spore size and shape in five single-spore cultures from a single isolate population. None of these characters contributed significantly to synapomorphies within the *Gigasporales*, being homoplastic with a consistency index (CI) below 0.4. Character 14 was synapomorphic for the genus *Dentiscutata*, although the CI was also below 0.4.

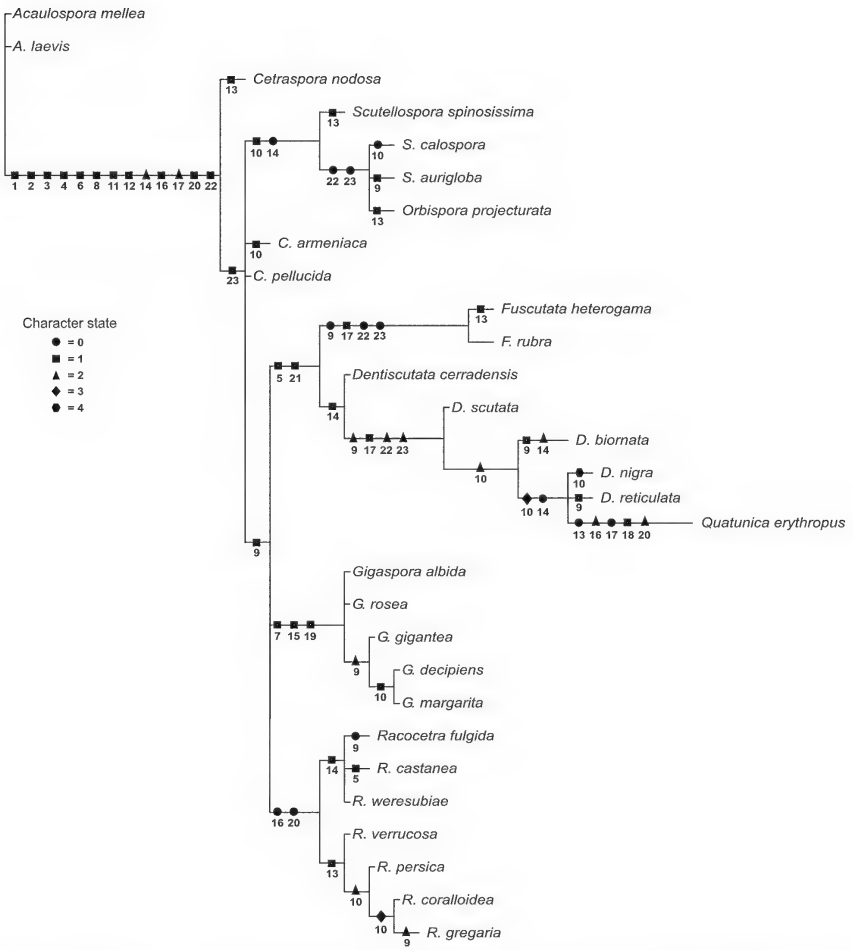

FIG. 4. Phylogenetic reconstruction of the *Gigasporales* based on 23 morphological characters. (Tree length = 63 steps; Consistency Index = 0.49; Retention Index = 0.77).

Some characters (7 and 19) that serve to separate *Gigaspora* from other gigasporalean taxa do not help solve the polyphyletic former *Scutellospora* groups. Thus, of 23 characters used by Morton & Msiska (2010), just ten are phylogenetically helpful in separating monophyletic groups. Most of these characters are related to germination shield, hyphal color, and germinal wall characteristics.

Despite the Morton & Msiska (2010) criticism of the LSU–SSU interdependence, they used two interdependent morphological characters

(16—number of germinal walls and 20—germination shield position) to group *Racocetra* taxa. In addition, this genus is grouped by the more ancestral character state (as coded by the authors) that clearly represents a symplesiomorphy, which does not provide evidence of common ancestry (Wiley et al. 1991). We conclude that Morton & Msiska (2010) erred when coding these character states.

Evolutionary line in the *Gigasporales*

Ontogenetic data (Franke & Morton 1994; Morton 1995; Bentivenga & Morton 1995, 1996) led readers to believe that *Gigaspora* might be basal to the former *Scutellospora*. However, several molecular studies (Oehl et al. 2008, 2010, 2011b, Goto et al. 2010; 2012) demonstrated that the former *Scutellospora* evolved first in the *Gigasporales*. The report of 400 million year-old fossil specimens with germination shields by Dotzler et al. (2006) suggested that the presence of germination shield is ancestral in *Gigasporales*. The low number of gigasporoid species related to scutellosporoid (sensu lato) species also suggests that *Gigaspora* diverged after other members. Finally, we understand the capacity of repeated germination of *Gigaspora* species (Maia & Kimbrough 1993) from ≤1000 germ warts randomly distributed on the spore wall inner surface as a evolutionary progression compared with single germination events from 1–24 germ tube initiations found on the germination shield periphery of scutellosporoid (sensu lato) species.

The molecular phylogenetic trees suggest that spore wall loss has occurred three times during the evolutionary history of *Gigasporales* [*Scutellospora*, *Cetraspora* (3) → *Racocetra*, *Intraornatospora* (2) → *Gigaspora* (1)], while at least once an additional wall was acquired [*Dentiscutata* (3) → *Quatunica* (4)]. However, even the germinal wall was lost in *Gigaspora* leaving only the innermost warty germination layer, and germination in this genus is related to this specific layer. *Intraornatospora* with one germinal wall with a rudimentary appearing germination shield (Goto et al. 2012) is close phylogenetically to *Gigasporaceae*. This genus forms a particular ornamentation on the inner surface of the outer spore wall (Goto et al. 2009) in the form of tubercular projections resembling the germ warts in *Gigaspora*. Since *Intraornatospora* germination has not yet been observed, it is not possible to infer whether germination occurs from the germination shield or tuberculate projections.

The increasing complexity of the germination shield structure can be observed in the gigasporalean evolutionary line. Clearly, the simple shield states (germ orbs = mono-lobed, germ violins = bi-lobed) are ancestral. The presence of color in the germination shield is a derivative state (shared by *Dentiscutataceae* species and the related *Paradentiscutata*), whereas this structure is clearly hyaline in the basal branch (*Orbispora*, *Scutellospora*).

Final considerations

The classification proposed by Morton & Msiska (2010) was based solely on morphological data that were not correctly interpreted. The authors did not consider their own molecular phylogenetic data, which supported almost all families and genera proposed by Oehl et al. (2008). Moreover, the *Gigasporales* classification suggested by Morton & Msiska (2010) does not reflect natural groups and leaves *Scutellospora* polyphyletic. Thus, we do not accept the analysis and assumptions by Morton & Msiska (2010), who misinterpreted some morphological characters, leading to misanalysis in the morphological phylogeny of the *Gigasporales.*

The low number of informative morphological characters in *Gigasporales* (until now) have not permitted a reliable morphology-based phylogeny. While we work to find more informative phylogenetic characters and reconstruct a reliable evolutionary history for *Gigasporales,* we should investigate the gigasporalean phylogeny using molecular tools.

Our current phylogenetic analyses demonstrate that all families and almost all genera proposed by Oehl et al. (2008) are monophyletic. Molecularly, generic relationships in the *Dentiscutataceae* are not yet completely understood but will be clarified once sequences of more species from this family are available. We believe that the classification by Oehl et al. (2008; 2011b) with implementation of Goto et al. (2012) is reliable and indicate the need for clarification of generic relationships in the *Dentiscutataceae.*

Acknowledgments

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Pleurotus eryngii var. *elaeoselini*, first record from Romania

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Abstract — *Pleurotus eryngii* var. *elaeoselini*, on *Laserpitium latifolium* roots, is reported in Romania for the first time. A description and illustrations of this variety are provided.

Key words — taxonomy, *Pleurotaceae*, *Apiaceae*

Introduction

Pleurotus eryngii var. *elaeoselini* was described by Venturella et al. (2000) from Sicily (Italy), growing on *Elaeoselinum asclepium* (L.) Bertol. subsp. *asclepium*. Subsequently, Venturella (2002) reported the variety from Spain, associated with other plants in *Apiaceae: Thapsia villosa* L. and *Elaeoselinum gummiferum* (Desf.) Tutin. Unpublished data from one co-author (G. Venturella) widen the distribution (i.e. France, Switzerland, Malta, Slovenia and Ukraine) of this taxon (Fig. 1). In Romania, data on the *P. eryngii* species complex are scarce, and only *P. eryngii* (DC.) Quél. on *Eryngium* spp. roots has been reported (Sălăgeanu & Sălăgeanu 1985). Identification of *P. eryngii* var. *elaeoselini* in Romania, collected on *Laserpitium latifolium* roots, adds new data to the existing knowledge of this fungus. *Laserpitium latifolium* is also known as a host for *P. eryngii* var. *eryngii* (Zervakis & Balis 1996). Zervakis et al. (2001), discussing the occurrence of *Pleurotus nebrodensis* (Inzenga) Quél. on *L. latifolium*, stated that *P. nebrodensis* should be associated only with *Cachrys ferulacea* (L.) Calest.

Materials & methods

The *P. eryngii* var. *elaeoselini* basidiomata, collected during 2006–11 in the Stânişoarei Mountains (Eastern Carpathians), Romania, were described and photographed in the habitat. The material was preserved by drying and deposited in the Herbarium of the Alexandru Ioan Cuza University of Iaşi (I). Duplicates of I-137264 and I-137266 are kept

FIGURE 1. Distribution of *Pleurotus eryngii* var. *elaeoselini* in Europe. ES (Spain), FR (France), CH (Switzerland), IT (Italy), MT (Malta), SI (Slovenia), RO (Romania), UA (Ukraine).

in Herbarium Mediterraneum Panormitanum (PAL). For microscopical examination, sections were mounted in 5% KOH and Congo red solution. The species was monitored during annual field trips in 2007–11.

Taxonomy

Pleurotus eryngii* var. *elaeoselini Venturella, Zervakis & La Rocca, Mycotaxon 76: 420 (2000) FIG. 2

BASIDIOMATA fleshy, 40–110 mm high. PILEUS 40–120 mm in diameter, hemispherical to convex when young, low convex to depressed when mature; whitish, white-cream to light beige, with alutaceous tones; smooth, often lacerated in small appressed areolae; margin deeply involute in young basidiomata, then straight, sometimes lobate. LAMELLAE deeply decurrent, arcuate, up to 10 mm broad, in young basidiomata with evident anastomoses on the stipe, whitish to light cream, sometimes grayish-white in young basidiomata, edge entire, concolorous. SPORE DEPOSIT white.

STIPE 25–85 × 6–20 mm, central to eccentric, concolorous with the pileus, filled and firm, irregularly cylindrical, straight to slightly curved, base attenuate

FIGURE 2. *Pleurotus eryngii* var. *elaeoselini* basidiomata in habitat (Photo: V.C. Chinan).

and sometimes radicating, lightly pruinose in the young basidiomata, then smooth, glabrous. CONTEXT compact, fibrous, white in pileus and stipe, odor and taste fungoid.

BASIDIA 4-spored, 34–48 × 8–10 µm, sterigmata 4–5 µm. BASIDIOSPORES 8–12(–13) × 4–5.5 µm, cylindrical-ellipsoid, apiculate, smooth, hyaline; cheilocystidia 40–60 × 8–12 µm, club-shaped.

HABITAT/SUBSTRATE/PHENOLOGY. Mountain meadow (alt. 620–750 m), calcareous soil, on *Laserpitium latifolium* roots, single or in groups, autumn (September–October).

SPECIMENS EXAMINED: **ROMANIA, EASTERN CARPATHIANS**: Stânișoarei Mountains, Neamț County, near Potoci village, mountain meadow, GPS: 46°56′14.55″N 26°07′43.86″E, alt. 620–750 m, on *Laserpitium latifolium* L. roots (*Apiaceae*), 6 Oct. 2006, 10 Oct. 2007, 19 Oct. 2008, 17 Sept. 2010, 13 Oct. 2011, coll. V. Chinan (I 137262; I 137263; I 137264, PAL 001/ROM; I 137265; I 137266, PAL 002/ROM).

Discussion

Pleurotus eryngii var. *elaeoselini* is a rare taxon in Europe. In Romania it was found on *L. latifolium* roots in a meadow in the Stânișoarei Mountains (Eastern Carpathians). This is the first record of this variety for Romania and the Carpathian Mountains. Annual field trips, carried out between 2007 and 2011, confirmed its presence in the same place every year from September to October. Although it was first found in Italy only on *E. asclepium* subsp. *asclepium* (Venturella et al. 2000), data from Spain (Venturella 2002) show that

this variety is linked to more than one species in the *Apiaceae.* The Romanian collections of *P. eryngii* var. *elaeoselini* on *L. latifolium* confirm this conclusion.

Zervakis et al. (2001) previously discussed the presence of *Pleurotus* on *Laserpitium* spp., noting that *Pleurotus* strains growing on *Laserpitium* spp. in central Europe and northern Italy show identical microscopic characteristics with *P. eryngii* var. *elaeoselini* and that these differ from *P. nebrodensis.*

The macroscopic and microscopic attributes of the Romanian specimens fit the description by Venturella et al. (2000). Confusion with *P. nebrodensis* is excluded, as its host plant, *C. ferulacea,* is not known in Romania.

The Romanian population of *P. eryngii* var. *elaeoselini* apparently fruits only in autumn, during September and October. In Italy, it is reported fruiting in both autumn and spring (Venturella et al. 2000), while in Spain the variety has been found only in spring (Venturella 2002).

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The authors wish to thank Vladimír Antonín (Czech Republic), Georgios I. Zervakis (Greece), and Shaun Pennycook (New Zealand) for critically reviewing the manuscript.

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MYCOTAXON

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A new species of *Hypoderma* with periphysoids

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Abstract — *Hypoderma mirabile*, a new species found on fallen leaves of *Litsea elongata* in China, is described and illustrated. This taxon is distinctive within the genus by the presence of periphysoids, unbranched curved paraphyses, and a unique covering stroma.

Key words — foliicolous fungi, morphological characteristics, taxonomy

Introduction

Hypoderma De Not. is one of the earliest described genera of *Rhytismatales*. It was established by De Notaris (1847) and proposed as conserved name by Cannon & Minter (1983). Höhnel designated *Hypoderma rubi* (Pers.) DC. as the lectotype species of the genus in 1917, and this viewpoint was subsequently accepted (Darker 1967; Cannon & Minter 1986; Korf 1988). Johnston (1990) emphasised the significance of the developmental and structural characters of the ascomatal sterile tissues rather than the ascospore shape to revise and expand the concept of *Hypoderma.* This genus is characterized by the presence of a preformed dehiscence mechanism, subcuticular ascomata, paraphyses that are never swollen, and ascospores that vary in shape.

Hypoderma is the third-largest genus in the *Rhytismataceae* (*Rhytismatales, Leotiomycetes, Ascomycota*), and of the 54 species known worldwide, many are widely distributed in the temperate and tropical zones (Kirk et al. 2008). The study of *Hypoderma* in China began in 1924 with the description of a new taxon, *Hypoderma strobicola* f. *cunninghamiae* Keissl., subsequently raised to species rank in 1947 as *H. handelii* Petr. (Keissler 1924; Petrak 1947). Afterwards, 13 additional species have been reported from China (Teng 1963; Tai 1979; Lin & Hou 1994; Lin et al. 2004; Liang et al. 2005; Hou et al. 2005, 2007; Hou & Piepenbring 2006; Wang et al. 2007).

Up to now, 5 species representing *Lophodermium* and *Coccomyces* in *Rhytismatales* have been recorded on *Litsea*, but no *Hypoderma* species has yet been reported on this plant genus (Farr & Rossman 2012).

Materials & methods

Macroscopic appearance was described under the dissecting microscope at 10–50× magnification. After rehydration of reference material in water for ca 10 min, 8–15 µm thick sections of mature fruitbodies were cut using a freezing microtome (YD-202, Yidi Medical Appliance Factory, Jinhua, Zhejiang, China). Sections were mounted in lactic acid or cotton blue with pretreatment in water for the observations of ascomatal and conidiomatal outlines in vertical section. The colours of various structures were observed in water. Gelatinous sheaths surrounding ascospores and paraphyses were examined in water or 0.1% (w/v) cotton blue in lactic acid. Measurements were made from more than 30 asci, ascospores, and paraphyses for each specimen using material mounted in 5% KOH or Melzer's reagent. Line and point integrated illustrations of external shape and internal structures of fruitbodies were drawn using a microscopic painting device (Panasoianic XSJ-2, Japan).

The type specimen is deposited in the Forest Fungi Dried Reference Collection of Anhui Agricultural University, China (AAUF).

Taxonomy

Hypoderma mirabile Y.R. Lin & C.T. Zheng, **sp. nov.** FIGS 1–5

MycoBank MB 800352

Differs from *Hypoderma rubi* and *H. cordylines* by the presence of periphysoids, smaller asci and ascospores, unbranched, curved paraphyses, and a distinct covering stroma.

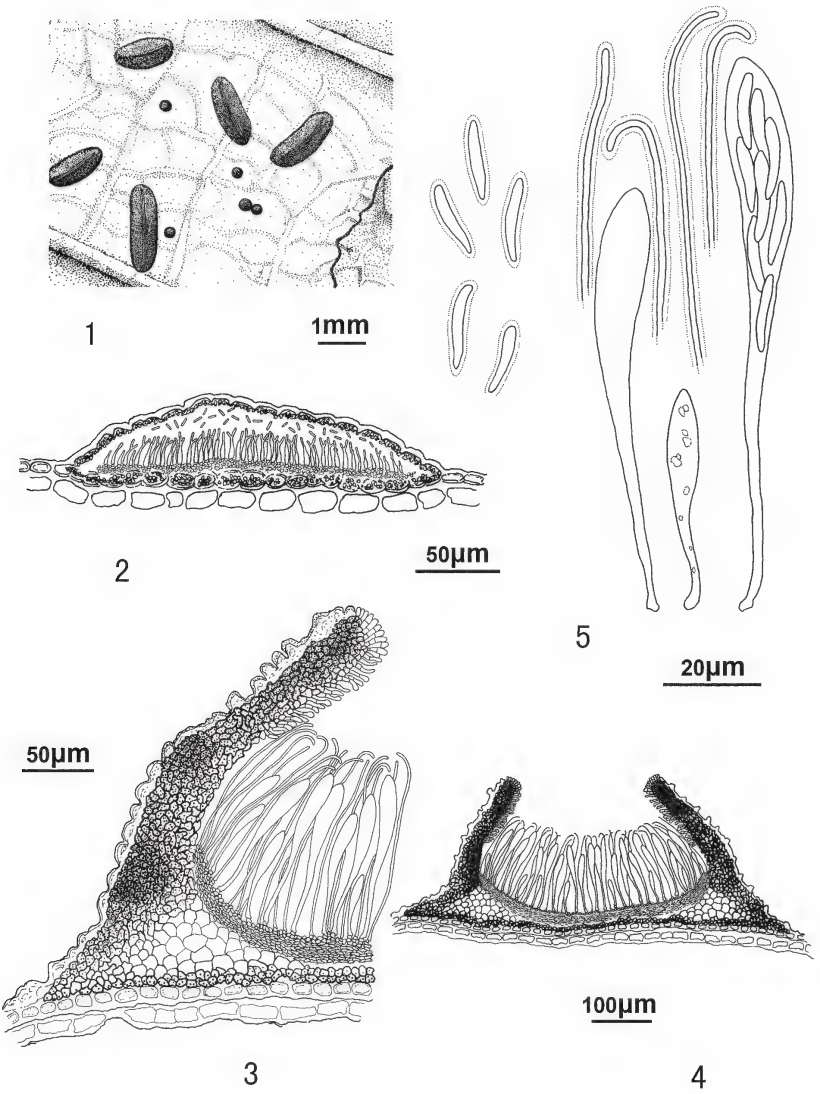
TYPE: China, Anhui, Tiantangzhai, Xibianwa, alt. 950 m, on fallen leaves of *Litsea elongata* (Wall. ex Nees) Benth. & Hook. f. (*Lauraceae*), 7 June 2007, J.J. Han & Y.R. Lin 2197 (**Holotype**, AAUF 68305).

ETYMOLOGY: *mirabile* (Latin = fantastic), referring to the morphological peculiarity of this fungus which has unique periphysoids.

COLONIES on both sides of leaves, forming distinct irregular, yellow-brown paler areas, 7–12 mm diam.

ZONE LINES somewhat frequent, dark brown, thin, surrounding or partly surrounding the pale areas, not always conspicuous.

CONIDIOMATA on both sides of leaves, scattered, occasionally coalescent. In surface view, conidiomata 150–250 µm diam., rounded or subrounded, somewhat raising the substratum surface, dark brown to black, but lighter coloured in immature conidiomata, with a surrounded black perimeter line, discharging spores through an inconspicuous central ostiole. In vertical section, conidiomata subcuticular. UPPER WALL poorly developed, composed of textura epidermoidea with tiny, thin-walled cells. BASAL WALL 3–7 µm thick, comprised of textura globulosa-angularis. SUBCONIDIOGENOUS LAYER



Figs 1–5. *Hypoderma mirabile* on *Litsea elongata*. 1. Fruitbodies and a zoneline observed under a dissecting microscope. 2. Conidioma in vertical section. 3. Portion of ascoma in median vertical section. 4. Ascoma in median vertical section. 5. Paraphyses, asci, and ascospores.

ca 6 µm thick, consisting of 3–4(–5) layers of hyaline, thin-walled angular cells. Conidiogenous cells 8–14 × 2–3.5 µm, cylindrical, tapering towards the apex, proliferating sympodially or occasionally proliferating percurrently. Conidia 4–6 × 1–1.5 µm, cylindrical, hyaline, unicellular.

Ascomata in similar positions to conidiomata on the substratum, scattered over the pale areas. In surface view, ascomata 1000–1700 × 500–650 µm, elliptical to elongated-elliptical, straight or somewhat curved, ends rounded or obtuse, black, slightly raising the substratum surface, opening by a single longitudinal split 3/4–4/5 the length of the ascoma. Lips moderately developed. In median vertical section, ascomata subcuticular. Covering stroma 20–30 µm thick near the opening, increasing to 50–55 µm thick not far away from the base, composed of dark brown textura angularis-epidermoidea with thick-walled cells 5–8 µm diam., connecting to the basal stroma. Lips with a layer of cells 7–14 × 3–4 µm, cylindrical, thin-walled, hyaline, 0–1(–2)-septate. Periphysoids lined on the inner face of the covering stroma, 15–18 × 2–3 µm, cylindrical, straight or curved, frequently swelling at the top, hyaline, 0–2-septate. Basal stroma 5–8 µm thick, dark brown, consisting of 1–2 layers of angular, thick-walled cells. Subhymenium moderately developed, 10–22 µm thick, comprised of hyaline textura porrecta with a region of colourless, gluey textura angularis with thin-walled cells 11–14 µm diam. at the edge of the ascoma. Paraphyses 125–140 × 1–1.5 µm, filiform, aseptate, unbranched, often curved but not swollen at the apex, covered with a 0.5–1 µm thick gelatinous coating. Asci ripening sequentially, 80–120 × 8.5–12 µm, cylindrical-clavate, long-stalked, thin-walled, apex rounded, without circumapical thickening, J–, 8-spored. Ascospores biseriate or irregularly arranged, confined to upper half of the ascus, 12–19 × 2.2–3 µm, cylindrical to cylindrical-clavate or clavate, hyaline, aseptate, with a gelatinous sheath ca 1 µm thick.

Host species, habitat and distribution: Producing conidiomata and ascomata on fallen leaves of *Litsea elongata*. Known only from the type locality, Anhui Province, China.

Comments — The periphysoidal layer is a structure similar to the lip cells present in some other species of *Rhytismatales*. This structure supposedly helps the covering stroma open when wet and close upon drying (Sherwood 1980). Except for a few *Coccomyces* and *Lophodermium* species (Johnston 1986; Sherwood 1980; Spooner 1990, 1991; Lin 1998; Lin et al. 2002), most rhytismatalean taxa, especially *Hypoderma* species, lack periphysoids. We recognize *H. mirabile* as a new species mainly due to its obvious periphysoidal layer.

Hypoderma rubi, which produces macroscopically similar ascomata, conidiomata, and zone lines and similarly shaped asci and ascospores, differs from *H. mirabile* in its covering stroma without periphysoids and sharply

narrowing from the edge of the opening, septate branched paraphyses, asci (87–130 × 12–14.5 μm), and ascospores [(16–)20–24(–30) × 2.5–3.5 μm] lacking an obvious gelatinous sheath (Cannon & Minter 1986).

The similar *H. cordylines* P.R. Johnst. is distinguished from *H. mirabile* by its covering stroma composed of angular cells that thins gradually or more or less abruptly toward the outside ascomal edge, well-developed lips, loosely circinate paraphyses tips, larger asci (90–140 × 11–16 μm) slightly thickened at the apex, uniform elliptic ascospores, the absence of an upper wall in the conidiomata, and much longer conidia (5–9 μm) (Johnston 1990).

From our observations, it seems likely that this new species is a weak parasite or a saprobe.

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Yeast species from soil and fallen leaves new for the mycobiota of Israel

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Abstract — We investigated the species diversity of yeasts from soil and fallen leaves of Israel based on sequencing of the D1/D2 domain of 26S rDNA. Seven new yeast records found for Israel were *Apiotrichum nothofagi, Cryptococcus carnescens*, *C. phenolicus*, *C. terreus*, *Komagataella pastoris, Rhodosporidium lusitaniae*, and *Schwanniomyces occidentalis*.

Key words — *Ascomycota*, *Basidiomycota*, Middle East, molecular methods

Introduction

The biodiversity of yeasts in Israel has not been studied well. There has not been any critical research regarding the biodiversity of this group of fungi. One thorough investigation was done at the "Evolution Canyon" microsite in Mount Carmel by Nagornaya et al. (2003). Some selective studies were dedicated to the biodiversity of yeasts in hypersaline water habitats (Butinar et al. 2005). *Yarrowia lipolytica* was found together with *Rhodotorula glutinis* on salt-excreting leaves of *Atriplex halimus* plants in the central Negev Highlands of Israel (Zvyagilskaya et al. 2001). Many yeast species were collected in vineyards in the Coastal Plain of Israel (Zahavi et al. 2002), and *Metschnikowia fructicola* was first described from grape surfaces in Israel (Kurtzman & Droby 2001). *Metschnikowia reukaufii* was isolated from pollen of *Asclepias syriaca* (Eisikowitch et al. 1990). *Clavispora lusitaniae* was first described from citrus essence in Israel (Rodrigues de Miranda 1979). Several yeast species have been recorded on mites, in citrus products and organic acid media, on *Zea mays*, and on grapefruit (Smith 1986, Steiman et al. 1997, Schena et al. 2000, Boekhout et al. 2003, Savchenko et al. 2010, Kurtzman et al. 2011).

Forty-two yeast species are reported in the literature from Israel: 24 ascomycetes (in 15 genera and eight families) and 18 basidiomycetes (in nine genera and eight orders).

We investigated yeast species diversity of soil and fallen leaves based on sequencing of the D1/D2 domain of 26S ribosomal DNA in order to achieve better insight into the biodiversity of yeast species in Israel.

Materials & methods

Collection site

Soil and fallen leaves samples were collected in En Ziwan, 33°11′09″N 35°79′10″E, Golan Heights; Carmel National Park, 32°75′00″N 35°02′70″E, Carmel Mount; and Kefar Kisch, 32°65′32″N 35°45′06″E, Lower Galilee.

Isolation techniques

To isolate the yeasts from soil and fallen leaves, 10 g of each sample was diluted in 100 ml of sterile double-distilled water and placed on the orbital shaker for one hour at 150 rpm at room temperature. After shaking, each sample was diluted decimally; 0.15 ml of each dilution was spread on acidified yeast-malt (YM) agar (1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, and 2% agar) pH 3.7–3.8, or YM agar containing chloramphenicol (0.01%) and sodium propionate (0.2%) for suppression of bacterial and mold growth. The plates were incubated at 25°C for 3–7 days. Representative colonies of each morphology type were picked and streaked onto new YM agar plates. The purity of yeast cultures was ascertained using light microscopy. Color names follow Rayner (1969). The yeast cultures were stored at 4°C in YM agar plates. They are available from culture collection of the Institute of Evolution, University of Haifa (HAI, Haifa, Israel). For long-term preservation they were frozen at –80°C in 25% glycerol YM medium.

Molecular-biological methods

Yeasts were identified taxonomically using molecular-biological methods. DNA was extracted according to Hoffman & Winston (1987) with Gottschling (http://labs.fhcrc.org/gottschling/Yeast%20Protocols/qgprep.html) and Dunham (http://dunham.gs.washington.edu/MDyeastDNAprep.htm) modifications. DNA concentration was measured using the NanoDrop®ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, Delaware, U.S.A.). The concentration of DNA was adjusted to 10 ng/µl for further applications. Diluted DNA (10ng/µl) was used as a PCR template. The D1/D2 domain of the large subunit (26S) of ribosomal DNA (rDNA) was amplified on GeneAmp® PCR System 9700 (Applied Biosystems, USA) using primers NL1 (5'-GCATATCAATAAGCGGAGGAAAAG) and NL4 (5'-GGTCCGTGTTTCAAGACGG) (O'Donnell 1993), and ABgene® Thermo Scientific 2X ReddyMix PCR Master Mix w/1.5 mM $MgCl_2$ (Applied Biosystems, California, USA). PCR cycling conditions included initial denaturation (95°C for 5 min), 5 extension cycles (94°C for 45 s, primer annealing at 55°C for 45 s, primer extension at 72°C for 1 min), 29 denaturation cycles (95°C for 30 s, primer annealing at 60°C for 30 s, primer extension at 72°C for 1 min), and a final elongation step at 72°C for 10 min. PCR products were visualized in 1.5% agarose gel, stained with GelRedTM Nucleic Acid Gel Stain, 10,000× in water (Biotium, Inc.,

Hayward, California), and viewed under UV. PCR products were cleaned using an ExoSAP-IT (USB Corp.) following the manufacturer's instructions.

The PCR products were sent to the Technion (Israel Institute of Technology, The Bruce Rappaport Faculty of Medicine) Medicine Lab for sequencing, which was performed on 3130xl Genetic Analyzer (Applied Biosystems, USA) using the PCR primers and Big Dye® Terminator v 1.1 Cycle (Applied Biosystems, USA). We identified the yeast species in a BLAST search. BLASTN 2.2.26+ program was to align sequences and compare them with type strains, percentage of query coverage, and max identity (Zhang et al. 2000). All sequences were registered in GenBank.

Results

Analysis of the 26S rDNA D1/D2 sequences revealed seven yeast species not previously reported for Israel, of which two are ascomycetes (*K. pastoris*, *S. occidentalis*) and five are basidiomycetes (*A. nothofagi*, *C. carnescens*, *C. phenolicus*, *C. terreus*, *R. lusitaniae*).

Apiotrichum nothofagi C. Ramírez & A.E. González, Mycopathologia 88(2–3): 76 (1984) [as "*Apiotricum*"]. FIG. 1

TYPE: Chile, Futrono, in evergreen rainy Valdivian forest, from decayed wood of *Nothofagus obliqua*, 1980, A. González (IJFM 6018).

≡ *Rhodotorula nothofagi* C. Ramírez & A.E. González, Mycopathologia 91(3): 171 (1985), nom. illegit. [superfluous sp. nov., based on same type].

≡ *Rhodotorula nothofagi* (C. Ramírez & A.E. González) Roeijmans, Eijk & Yarrow, Mycotaxon 35(2): 406 (1989), nom. illegit. [superfluous, later homonym].

CELLS after 3 days growth on YM agar at 25°C subglobose to ellipsoid, 3–4 × 6–11 µm, occurring mostly singly or in pairs. Budding predominantly polar. COLONIES white and smooth.

SPECIMENS EXAMINED – **ISRAEL. CARMEL MOUNT**, Haifa, Carmel National Park, 32°75′18″N 35°02′69″E, 18.XII.2010, from fallen needles of *Pinus halepensis*, leg. D.M. Gotman (HAI-Y-35; GenBank JQ581048); **LOWER GALILEE**, Kefar Kish, 32°65′32″N 35°45′06″E, 22.XII.2010, from fallen leaves of *Ceratonia siliqua*, leg. D.M. Gotman (HAI-Y-81; GenBank JQ581049).

COMMENTS – *Apiotrichum nothofagi* belongs to the *Curvibasidium* clade of *Microbotryomycetes*. A single strain IJFM 6018 (CBS 8166) was isolated in 1980 from decayed wood of *Nothofagus obliqua*. In 1984, Ramírez & González described a new taxon, *Apiotrichum nothofagi*, citing IJFM 6018 as type culture. The following year, they published *Rhodotorula nothofagi* as a new species, also citing IJFM 6018 as type culture, rendering *R. nothofagi* a superfluous and illegitimate name (ICBM [Vienna Code] Art. 52.1). Apparently unaware that *R. nothofagii* had already been validly published by Ramírez & González, Roeijmans et al. (1989) recombined *A. nothofagi* as *Rhodotorula nothofagi*, a comb. nov. that is also a superfluous and illegitimate later homonym. We cite this taxon here under its only legitimate name, *Apiotrichum nothofagi*, but it

requires a nom. nov. in *Rhodotorula*, since both existing *Rhodotorula* names are illegitimate.

Distribution – Europe, North America, South America (Gadanho et al. 2003, Maksimova & Chernov 2004, Villa-Carvajal et al. 2004, Langdon et al. 2005, Rodrigues et al. 2009, Golubev & Tomashevskaya 2010).

Cryptococcus carnescens (Verona & Luchetti) M. Takash. et al., Int. J. Syst. Evol. Microbiol. 53(4): 1192 (2003) Fig. 2

Cells after 3 days growth on YM agar at 25°C single, ellipsoidal or subglobose, 3–7 µm; budding monopolar. Colonies [D]-colored, smooth.

Specimen examined – **ISRAEL. Golan Heights**, En Zivan, 33°11′09″N 35″79′10″E, 11.I.2011, from fallen leaves of *Quercus boissieri*, leg. D.M. Gotman (HAI-Y-183; GenBank JQ317682).

Comments – *Cryptococcus carnescens* belongs to the *Tremellales* (*Tremellomycetes*).

Distribution – Europe, Asia, Antarctica (Gadanho et al. 2003, Wuczkowski et al. 2005, Butinar et al. 2007, Connell et al. 2008, Golubev & Tomashevskaya 2010, Li et al. 2010, Loque et al. 2010, Kurtzman et al. 2011, Baleiras-Couto et al. 2012).

Cryptococcus phenolicus Á. Fonseca et al., Can. J. Microbiol. 46(1): 24 (2000) Fig. 3

Cells after 3 days growth on YM agar at 25°C single, globose, 4–8 µm in diam; budding monopolar. Colonies [B]-colored with glossy and smooth surfaces.

Specimen examined – **ISRAEL. Golan Heights**, En Zivan, 33°11′16″N 35″79′10″E, 11.I.2011, from grassland soil, leg. D.M. Gotman (HAI-Y-158; GenBank JQ581045).

Comments – This species belongs to the *Filobasidiales* lineage. Based on its ability to degrade phenol, *C. phenolicus* may be used to assimilate different aromatic compounds (Fonseca et al. 2000).

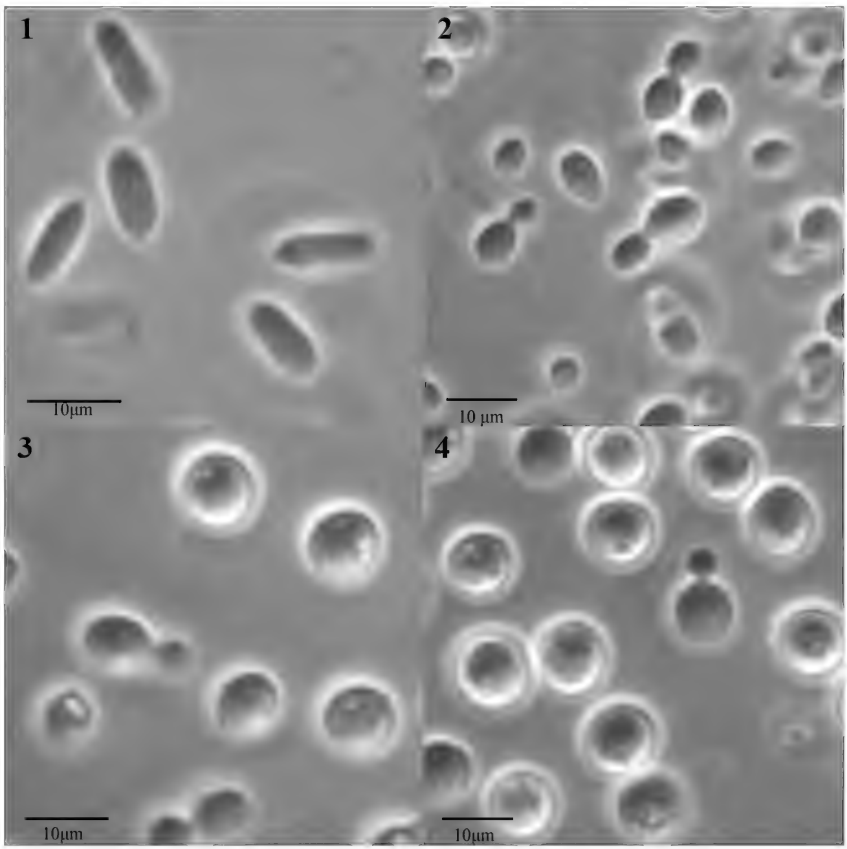
Distribution – Europe, Asia, South America, Africa (Hong et al. 2002, Cornelissen et al. 2003, Zachow et al. 2009, Vreulink et al. 2010, Mestre et al. 2011).

Cryptococcus terreus Di Menna, J. Gen. Microbiol. 11: 195 (1954) Fig. 4

Cells after 3 days growth on YM agar at 25°C single, mainly globose, 5–11 µm in diam; budding monopolar. Colonies [C/E]-colored, with glossy smooth surfaces.

Specimen examined – **ISRAEL. Golan Heights**, En Zivan, 33°11′16″N 35″79′10″E, 11.I.2011, from grassland soil, leg. D.M. Gotman (HAI-Y-162; GenBank JQ581046).

Comments – This species is also in the *Filobasidiales* lineage with *C. phenolicus* and was isolated from the same habitat. Apparently, *C. terreus* is a soil inhabitant. As with *C. phenolicus*, *C. terreus* can assimilate phenol and phenol-related compounds (Bergauer et al. 2005). *Cryptococcus terreus*, *C. fuscescens,* and *C. phenolicus* are the main cryptococcal soil inhabitants (Kurtzman et al. 2011).



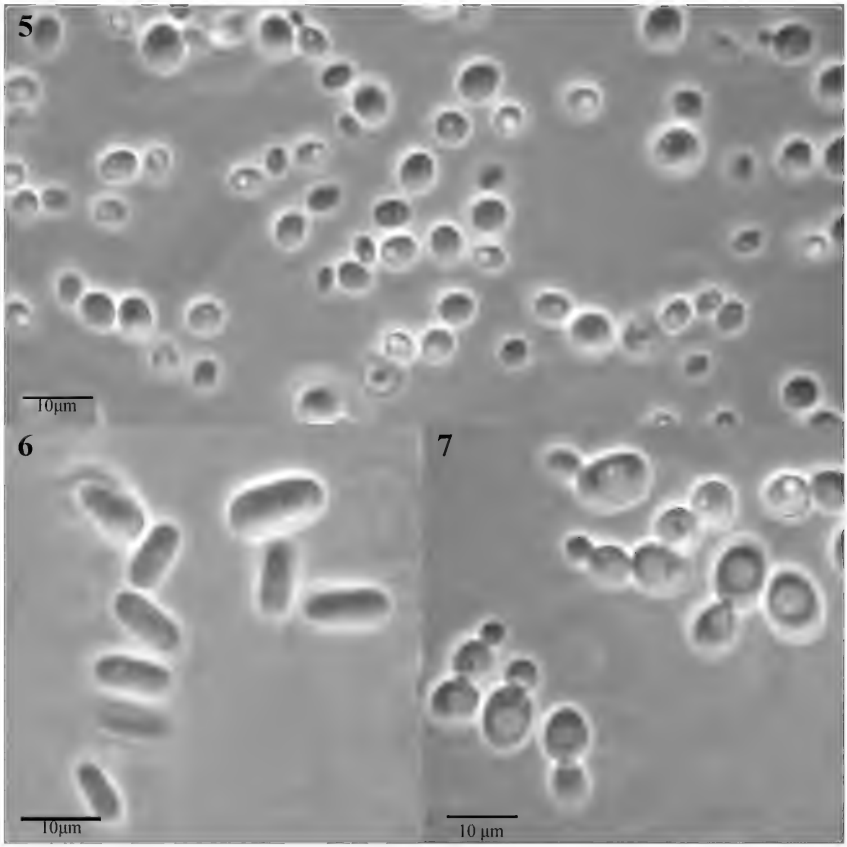
Figs 1–4. Yeast cells after two days of growing on YM medium, dark field light microscopy. 1. *A. nothofagi*; 2. *C. carnescens*; 3. *C. phenolicus*; 4. *C. terreus*. Scale bar = 10 µm.

Distribution – Europe, Asia, North America, South America, Oceania (Ba et al. 2000; Sláviková &Vadkertiová 2000; Hong et al. 2002, 2006; Maksimova & Chernov 2004; Mankowski & Morrell 2004; Bergauer et al. 2005; Langdon et al. 2005; Borelli et al. 2006; Lynch & Thorn 2006; Vishniac 2006; Rodrigues et al. 2009; Singh et al. 2009; Wang et al. 2009; Golubev & Tomashevskaya 2010; Mestre et al. 2011).

Komagataella pastoris (Guillierm.) Y. Yamada et al., Biosc., Biotechn., Biochem. 59(3): 444 (1995) Fig. 5

Cells after 3 days growth on YM agar at 25°C spherical to ovoid, 2–6 µm, occurring singly or in pairs; budding polar. Colonies white, mucoid.

Specimen examined – **ISRAEL. Carmel Mount**, Haifa, Carmel National Park, 32°75′03″N 35°02′77″E, 18.XII.2010, from soil under *Quercus calliprinos*, leg. D.M. Gotman (HAI-Y-67; GenBank JQ581044).



Figs 5–7. Yeast cells after two days of growing on YM medium, dark field light microscopy. 5. *K. pastoris*; 6. *R. lusitaniae*; 7. *S. occidentalis*. Scale bar = 10 µm.

Comments – *K. pastoris* represents *Phaffomycetaceae* in the *Saccharomycetales*. The major habitats of *K. pastoris* are tree exudates and decomposing wood. Phylogenetically, it is very close to *K. phaffii* and *K. pseudopastoris*, having no differences in fermentation and growth, but only in D1/D2 LSU rRNA gene sequences (Kurtzman 2005). They also were isolated from tree exudates and rotted wood. *K. pastoris* has a strong biotechnological potential as a recombinant protein production system (Heyland et al. 2010).

Distribution – Europe, North America, South America, Africa (Phaff & Knapp 1956; Faparusi 1981; Lachance et al. 1982, 1995; Spencer et al. 1995; Dlauchy et al. 2003).

Rhodosporidium lusitaniae Á. Fonseca & J.P. Samp., Syst. Appl. Microbiol. 15(1): 48 (1992) FIG. 6

CELLS after 3 days growth on YM agar at 25°C single, cylindrical to bacilliform, 3–4 × 9–11 µm; budding polar. COLONIES apricot colored, smooth.

SPECIMEN EXAMINED – **ISRAEL. CARMEL MOUNT**, Haifa, Carmel National Park, 32°75′00″N 35°02′70″E, 18.XII.2010, from fallen leaves of *Quercus calliprinos*, leg. D.M. Gotman (HAI-Y-58; GenBank JQ581047).

COMMENTS – *Rhodosporidium lusitaniae* is classified in *Sporidiobolales* (*Microbotryomycetes*).

DISTRIBUTION – Europe (Bergauer et al. 2005), where it is restricted to Austria, Israel, and Portugal.

Schwanniomyces occidentalis Klöcker, Meddn Carlsberg Lab. 7: 276 (1909) FIG. 7

CELLS after several days growth on YM agar at 25°C globose to ovoid, 5–11 µm in diam., singly or in pairs and small chains; budding polar. COLONIES white, smooth.

SPECIMEN EXAMINED – **ISRAEL. LOWER GALILEE**, Kefar Kish, 32°65′45″N 35°44′44″E, 22.XII.2010, from soil, leg. D.M. Gotman (HAI-Y-90; GenBank JQ581050).

COMMENTS – Almost all known strains of this species in the *Debaryomycetaceae* (*Saccharomycetales*) have been isolated from soil. *Schwanniomyces occidentalis,* which is regarded as an important "non-conventional" yeast with potential biotechnological value, has a good capacity for starch degradation. Several genetic models were developed for the production of heterologous proteins from *S. occidentalis* (Spencer et al. 2002).

DISTRIBUTION – Europe, Asia, North America (Capriotti 1957, Phaff et al. 1960, Urano et al. 2002, Sláviková &Vadkertiová 2003, Boby et al. 2008).

Discussion

Our research has revealed seven yeast species new for Israeli mycobiota. Four were found in soil (*C. phenolicus*, *C. terreus*, *K. pastoris*, *S. occidentalis*), one on fallen leaves of *Quercus boissieri* (*C. carnescens*), and one on fallen leaves of *Quercus calliprinos* (*R. lusitaniae*). *Apiotrichum nothofagi* was isolated both from fallen leaves of *Ceratonia siliqua* and from fallen needles of *Pinus halepensis*. Five species are new for the Middle East. Only *C. terreus* and *S. occidentalis* were previously recorded in Egypt (Youssef & Eldin 1998, El-Assal et al. 2011).

Worldwide, *Cryptococcus terreus* and *Schwanniomyces occidentalis* have been isolated from various soil types and have wide geographical distribution. The cosmopolitan *C. carnescens* has been found on different substrates all over the world. Besides soil, *C. carnescens* has been isolated from grape surface, seawater, marine microalgae, leaves, needle litter, and even from such extreme habitats as

glacial ice. Soil and leaf litter are the main habitats for *Rhodosporidium lusitaniae*, which may possibly inhabit the entire Mediterranean region in addition to Israel and Portugal; its connection with Mediterranean forest ecosystems can be traced. *Komagataella pastoris*, associated with tree exudates in deciduous forests, has been recorded in different parts of the world. In Israel it was found in soil (under oak), an unusual habitat for the species; yeast cells may possibly have entered the soil from decayed oak wood. The habitat of *A. nothofagi* is not so clear, as the species has been recorded only a few times, mainly associated with forest litter and decayed wood. This species has also been isolated from seawater and blueberry fruit.

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A new record of the desert truffle *Picoa lefebvrei* in Saudi Arabia

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Abstract — A new record of *Picoa lefebvrei* from Saudi Arabia is reported accompanied by notes on its taxonomy, ecology, and distribution.

Key words — truffle, drip irrigation, Arar

Introduction

Hypogeous mushrooms named desert truffles are commercialized in Saudi Arabia under the local name of faqaa [including khalasi, which belongs to *Terfezia* (Tul. & C. Tul.) Tul. & C. Tul., and zubaidi, which is used for species of *Tirmania* Chatin]. Local populations appreciate desert truffles for their nutritional and sensory qualities (Bokhary & Parvez 1988). *Picoa lefebvrei* or bird truffle (known locally as hober) was first reported by Bokhary & Parvez (1988; as *Phaeangium lefebvrei* Pat.) from Harrat al Harra in Al Jouf Province (northern Saudi Arabia). During winter and spring field excursions in the eastern sector of Northern Borders Province, the senior author (A. Bawadekji) discovered a new locality in Saudi Arabia for *P. lefebvrei*.

Materials & methods

The investigation was carried out from November to March during 2010–12. The habitat was recorded and the collected samples were identified by examining the peridium and gleba. Ascospores were examined in water under a Leica DMLB microscope. Nomenclature follows MycoBank (http://www.mycobank.org). The collection is deposited in the newly established Herbarium of the Department of Biological Sciences, Northern Borders University, Kingdom of Saudi Arabia (here cited as KSA); a duplicate is stored in the Herbarium Mediterraneum Panormitanum in Palermo (PAL).

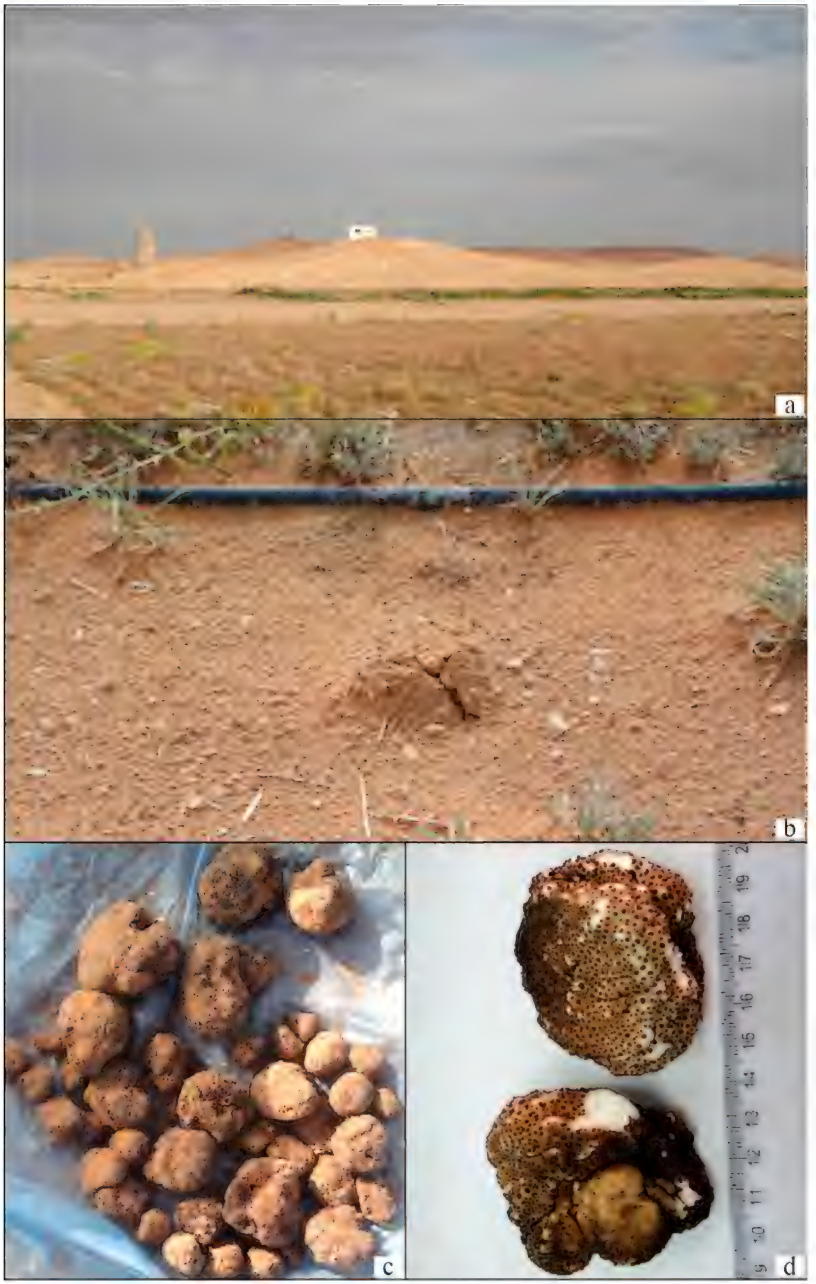

Fig. 1: *Picoa lefebvrei.*
a. Habitat of growth. b. A typical outgrowth of the soil. c. Ascomata. d. Detail of peridium.

Fig. 1: *Picoa lefebvrei*.
a. Ascus and ascospores. b. Peridial hyphae.

Species description

Picoa lefebvrei (Pat.) Maire, Ann. Mycol. 4: 332. 1906. Figs 1, 2

Ascomata irregularly globose, surface shining when ripe, hypogeous and semi-hypogeous, growing in groups, 0.5–8 cm diameter, brown-ochre, covered by numerous and small irregular warts, 1–2 mm wide, ca. 1 mm tall, sparse. Peridium 0.5 mm thick, pseudoparenchymatic, with angular-subglobose elements bearing septate hairs, ochre-brown to brown, filled with fine granules,

thick-walled, 100–120 × 8–10 µm. Cylindrical hyaline hyphae are present under the subglobose cells layer. Odour *Terfezia*-like, not as strong at the beginning of fruiting season (November to mid-January), becoming more intense as the air and soil temperatures increase. Gleba firm, whitish, veins more or less evident. Asci ellipsoidal, with 6–8 spores, 50–110 × 50–75 µm, encompassed in the trama, with a long peduncle, then subglobose and shortly pedunculate. Ascospores disorderly arranged in the ascus (Fig. 2), subglobose to broadly ellipsoidal, 25-30 × 20-25 µm, hyaline with a big guttule when unripe, then pale olive and covered by tiny, rounded, cyanophilous warts.

Specimens examined: **SAUDI ARABIA. Northern Borders Province**, Muayala Natural Reserve, 15 km south of Arar, 30°54′24″N 40°59′56″E, 552 m, on pastures, on calcareous and sandy soils with neutral pH reaction and low levels of organic matter, 10 Jan 2010 (provisional number KSA 001), 20 Dec 2011 (provisional number KSA 002), 13 Jan 2012 (PAL 001/KSA), coll. A. Bawadekji.

Ecology

Picoa lefebvrei fruits from January to April in North Africa and Middle East during years of adequate rainfall (Alsheikh & Trappe 1983). In Kuwait the species is confined to gypsiferous and calcareous gravelly deserts, where it is dug out and eaten by several species of migrating birds (Alsheikh & Trappe 1983).

Bokhary & Parvez (1988) regarded some ecological factors as favorable for production of desert truffles. In particular they considered rainfall as important for fructification of *Terfezia* and *Picoa* species. Morte et al. (2009), who stressed the importance of irrigation in cultivating desert truffles, pointed out that irrigation should be applied first at the end of the summer during dry years when rainfall is less than 150 mm and next at the beginning of the fruiting season in very dry years. It is interesting to note that the collection sites for *P. lefebvrei* in Muayala Natural Reserve experience drip irrigation of ca. 150 mm of water per year, while the mean annual rainfall value for this locality is only 40 mm.

Discussion

Picoa lefebvrei was originally placed in the genus *Phaeangium* Pat. because of spore ornamentation and tomentose peridium not present in other *Picoa* species (Alsheikh & Trappe 1983). Ammarellou et al. (2011) through phylogenetic ITS and 28s rDNA sequence analyses demonstrated that *P. lefebvrei* belongs to the *Geopora–Tricharina* clade of the *Pyronemataceae* (*Pezizales*, *Ascomycota*), placing *Phaeangium lefebvrei* in *Picoa* Vittad. based on its close morphological and genetic relationship with the type species, *Picoa juniperis* Vittad. Our macro- and micro-morphological observations of *P. lefebvrei* are in accord with Ammarellou et al. (2011) and confirm its placement in *Picoa*.

The new collection locality for *P. lefebvrei* is 15 km south of the city of Arar, in Northern Borders Province. Because the locality reported by Bokhary & Parvez (1988) is 400 km away in Al Jouf Province, our new finding constitutes a significant extension to the distribution of *P. lefebvrei* in Saudi Arabia.

Furthermore, the ecological features of the investigated area help identify a correct and sustainable management protocol for establishing good practices for commercial cultivation of *P. lefebvrei* for trade and to halt desertification processes.

Acknowledgements

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Coccomyces hubeiensis, a new fungus of *Rhytismatales* from China

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Abstract —A new fungus found on fallen leaves of *Rhododendron erubescens* from the Shennongjia forestry region of Hubei Province, China, is described as *Coccomyces hubeiensis*. This species differs from *C. dentatus* by its asci with subtruncate-conical apices, paraphyses branched near the top, infrequent zone lines, and the absence of conidiomata. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words —*Rhytismataceae*, morphology, *Ericaceae*

Introduction

Coccomyces De Not., established by de Notaris in 1847, is now placed in the *Rhytismatales* (*Leotiomycetes*, *Ascomycota*) (Kirk et al. 2008). *Coccomyces* taxa are characterized by polygonal or more or less circular ascomata that open by several radiate or irregular splits, cylindrical to clavate asci, and filiform, long-fusiform, or clavate ascospores, often with gelatinous sheaths (Sherwood 1980; Cannon & Minter 1986; Johnston 1986, 2000; Spooner 1990; Lin et al. 1994; Jia et al. 2012). External shape, size, and ascomal openings, ascal shape and size, and the shape of paraphyses at the apex are regarded as the most important taxonomic characters (Johnston 1986; Lin 1998).

Twenty-five *Coccomyces* species have been reported in China (Korf & Zhuang 1985; Lin 1998; Hou et al. 2006, 2007; Jia et al. 2012; Zheng et al. 2012) since Teng (1934) first recorded *C. dentatus* on *Quercus* and *Castanea* and *C. delta* on *Lauraceae*. Recently, a new *Coccomyces* species was found on fallen leaves of *Rhododendron erubescens* in the Shennongjia forestry region, Hubei Province.

Materials & methods

Mature ascomata with macroscopic characteristics of *Rhytismataceae* were selected from the collected specimens. We observed external shape, size, color, the opening

mechanisms of ascomata, and zone line characteristics using a stereoscope with 10–50× magnification.

The fruitbodies were rehydrated in water for 10 min, and 10–15 μm thick sections of the ascomata were cut using a freezing microtome. Microscopic examinations were mounted in water, 5% KOH, Melzer's reagent, or 0.1% (w/v) cotton blue in lactic acid. For observing the outlines of ascomata in vertical section, sections were mounted in lactic acid or cotton blue with pretreatment in water. The gelatinous matrix surrounding ascospores and paraphyses was observed in water or cotton blue in lactic acid. The color of internal structures was observed in water. Measurements and drawings of asci, ascospores, and paraphyses were made using material mounted in 5% KOH or Melzer's reagent and from 30 ascospores, asci, and paraphyses for each specimen. Illustrations of external shapes and internal structures of the ascomata were prepared using the Panasoianic XSJ-2 microscope drawing device.

Taxonomy

Coccomyces hubeiensis Y.R. Lin & M.S. Yang, **sp. nov.** FIGS 1–5

MycoBank MB800351

Differs from *Coccomyces dentatus* by its branched paraphyses and asci with subtruncate-conical apices and from *C. cyclobalanopsis* by its lack of excipulum and periphysoids.

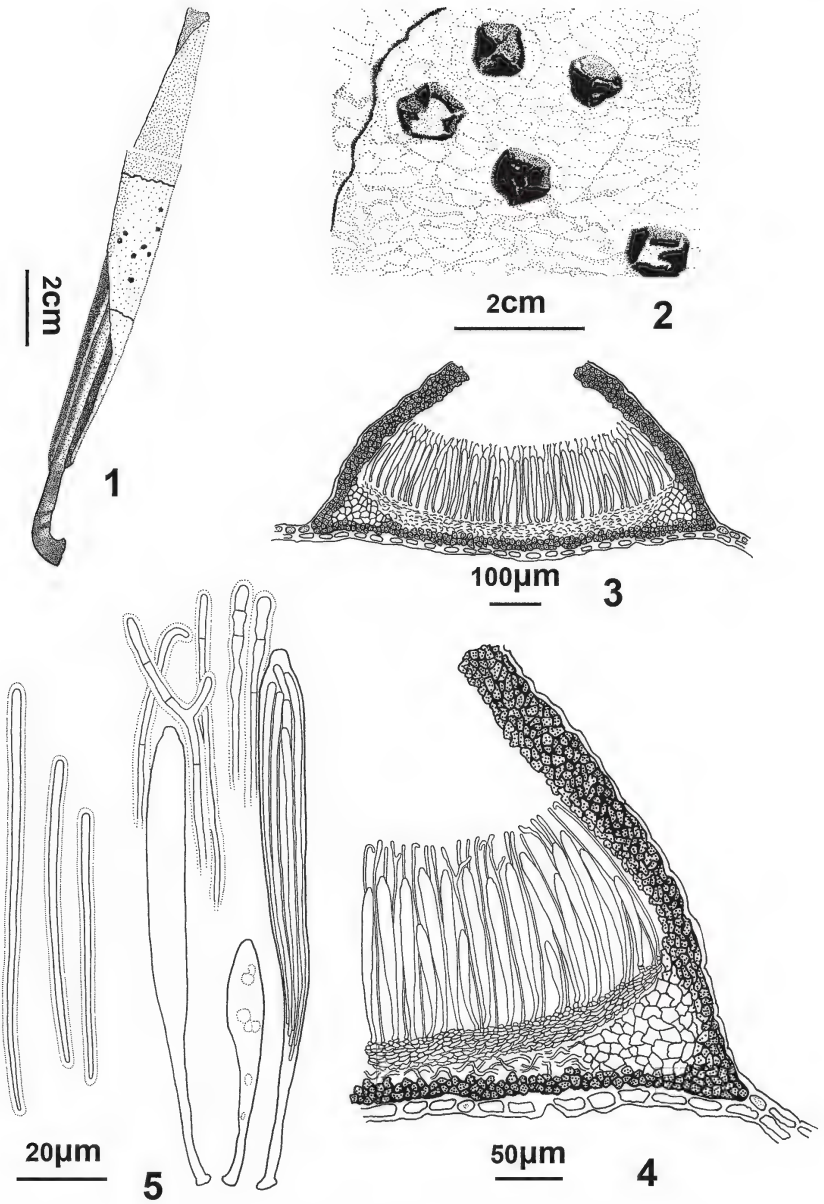
TYPE: China, Hubei, Shennongjia forestry region, Xiaolongtan, alt. 1300m, on fallen leaves of *Rhododendron erubescens* Hutch. (*Ericaceae*), 9 July 2010, Y.R. Lin, J.L. Chen & Q. Zheng 2385 (AAUF 68493).

ETYMOLOGY: *hubeiensis*, referring to the province where the specimen was collected.

ZONE LINES black-brown, infrequent, surrounding the bleached spots.

CONIDIOMATA not observed.

ASCOMATA on both sides of the fallen leaves, but predominantly on the upper side, sparsely scattered in light grey-white, subcircular or irregular bleached areas 15–25 mm diam. without obvious edges. In surface view, ascomata triangular to pentagonal, 570–950 μm diam., shining black, moderately raising above the surface of leaf but flat or slightly hollow in the centre portion, with an obvious preformed dehiscence mechanism, opening by 3–5 radial splits which extend nearly to the edge of ascoma to expose the light yellow-brown hymenium. Lips absent. In median vertical section, ascomata intraepidermal. COVERING STROMA 30–35 μm thick near the opening, composed of black-brown textura epidermoidea or angularis with thick-walled cells 4–7 μm diam., aliform cells mainly existing near the inner layer of the covering stroma, becoming thinner towards the edge gradually and extending to the basal stroma. EXCIPULUM absent. BASAL STROMA 10–18 μm thick, consisting of textura angularis with 2–3 rows of black-brown, thick-walled cells 4–6 μm diam. Colorless, thin-walled, large angular cells 6–12 μm diam. exist between the covering stroma and basal stroma. INTERNAL MATRIX STROMA poorly developed, comprised of hyaline, gelatinised textura intricata. SUBHYMENIUM 15–20 μm thick, composed of



Figs 1–5. *Coccomyces hubeiensis* on *Rhododendron erubescens*. 1. Habit on a leaf. 2. Detail of ascomata and a zone line. 3. Ascoma in median vertical section. 4. Portion of ascoma in median vertical section. 5. Paraphyses, asci and ascospores.

colorless textura porrecta-intricata. Paraphyses filiform, 90–150 × 1.5–2 μm, septate, the tips gradually swollen to 2.5–3 μm, rarely curved or branched, and sometimes forming epithecium above the asci, enveloped in mucus 0.8–1.2 μm thick. Asci ripening sequentially, cylindric-clavate, 80–120 × 7.5–10 μm, apex subtruncate-conical, without circumapical thickening, thin-walled, somewhat long-stalked, J–, 8-spored. Ascospores arranged in a fascicle, filiform, 65–102 × 1.5–1.8 μm, hyaline, aseptate, gradually tapered to the round distal end, with a gelatinous sheath ca 1 μm thick.

Host species & distribution: producing ascomata on fallen leaves of *Rhododendron erubescens*; known only from the type locality, Hubei, China.

Comments—*Coccomyces dentatus* is closely related to *C. hubeiensis* in the shape of asci and in the absence of lips, periphysoids, and excipulum, but differs in ascomata that are quadrate to hexagonal, black zone lines, the presence of abundant conidiomata, and paraphyses that are apically unbranched and do not form an epithecium (Sherwood 1980). *Coccomyces coronatus* is distinguished from the new species by its orbicular or polygonal ascomata, short-stalked larger asci (100–130 × 11–13 μm), and shorter and wider ascospores (60–80 × 2–2.5 μm) (Sherwood 1980). *Coccomyces hubeiensis* resembles *C. cyclobalanopsis* in the shape and size of ascomata, paraphyses, and ascospores as well as in the color of the exposed hymenium. However, *C. cyclobalanopsis* differs in having small abundant bleached spots, a covering and basal stroma consisting of a textura globulosa, and an extremely well-developed excipulum arising from the inner layer of the covering stroma (Lin et al. 2000).

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A microfungus from Costa Rica: *Ticosynnema* gen. nov.

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Abstract — *Ticosynnema carranzae* gen. & sp. nov., a new microfungus collected on the twig of an unidentified plant, is described and illustrated. It is distinguished by synnematous determinate conidiomata, monoblastic integrated determinate conidiogenous cells, and solitary, 3–4-septate, oblong, cylindrical to vermiform, brown conidia that secede rhexolytically.

Key words — anamorphic fungi, systematics, leaf litter

During an ascomycete-basidiomycete workshop field course at the VII Latin-American Mycological Congress, more than 100 samples of dead plant material colonized by anamorphic fungi were collected in a dry forest at the wildlife station in Santa Rosa National Park, Guanacaste, Costa Rica. Among these samples a conspicuous synnematous fungus was collected, which is herein described and illustrated.

Taxonomy

Ticosynnema R.F. Castañeda, Granados & Mardones, **anam. gen. nov.**

MycoBank MB800232

Differs from *Leuliisinea* by brown to black conidiomata and determinate conidiogenous cells, from *Endophragmiella* by synnematous conidiomata and determinate

conidiogenous cells, and from *Kramasamuha* by synnematous conidiomata and unbranched filaments.

Type species: *Ticosynnema carranzae* R.F. Castañeda et al.

Etymology: *Tico-*, is an indigenous word for Costa Rica, *-synnema*, referring to the type of conidioma of this anamorphic fungus.

Anamorphic fungi. Conidiomata on the natural substratum synnematous, scattered, determinate, dark brown to black. Mycelium superficial and immersed. Conidiophores macronematous, mononematous, erect, septate, loosely packed or compact, brown to dark brown. Conidiogenous cells monoblastic, integrated, determinate, terminal. Conidial secession rhexolytic. Conidia solitary, acrogenous, cylindrical, vermiform to oblong, with a conspicuous basal frill produced by the rhexolytic fracture of the wall of the conidiogenous cells, septate, foveate, smooth or verruculose, pale brown to brown. Teleomorph unknown.

Note: *Ticosynnema* superficially resembles *Leuliisinea* Matsush., but has percurrent proliferating, annellate conidiogenous cells and hyaline conidiomata. In fact, the two genera are similar only with respect to rhexolytic conidial secession (Matsushima 1985, Seifert et al. 2011). *Endophragmiella* B. Sutton and *Kramasamuha* Subram. & Vittal (Seifert et al. 2011) also produce conidia by rhexolytic fracture of the monoblastic conidiogenous cells, but both lack synnematous conidiomata. *Endophragmiella* has percurrent conidiogenous cell proliferations while such proliferations are absent in *Ticosynnema* and *Kramasamuha*. In addition, short sympodial or verticillate branches are present in *Kramasamuha* (Subramanian & Vittal 1973, Seifert et al. 2011).

Ticosynnema carranzae R.F. Castañeda, Granados & Mardones, **anam. sp. nov.** Figs 1,2

MycoBank MB800231

Differs from *Leuliisinea bambusicola* by having brown to black conidiomata and determinate conidiogenous cells, from *Endophragmiella* spp. by synnematous conidiomata and determinate conidiogenous cells, and from *Kramasamuha sibika* by synnematous conidiomata and unbranched filaments.

Type: Costa Rica, Guanacaste, Santa Rosa National Park, wildlife station, 10°52 N 85°35 W, on living twig of unidentified plant, 15 July 2011, coll. Dr. Aida Vasco Palacios, C11/51 (**Holotype,** HAL 2454 F).

Etymology: Latin, *carranzae*, named in honor of Dr. Julieta Carranza, a Costa Rican mycologist, for her contribution to Latin-American mycological progress and development.

Conidiomata on the natural substratum synnematous, determinate, scattered, erect, dark brown to black, 250–600 µm tall, 40–70 µm wide. Stipe composed of parallel hyphal filaments (conidiophores), multi-septate, straight, cylindrical, closely packed at the base and loosely towards the apex, black or dark brown

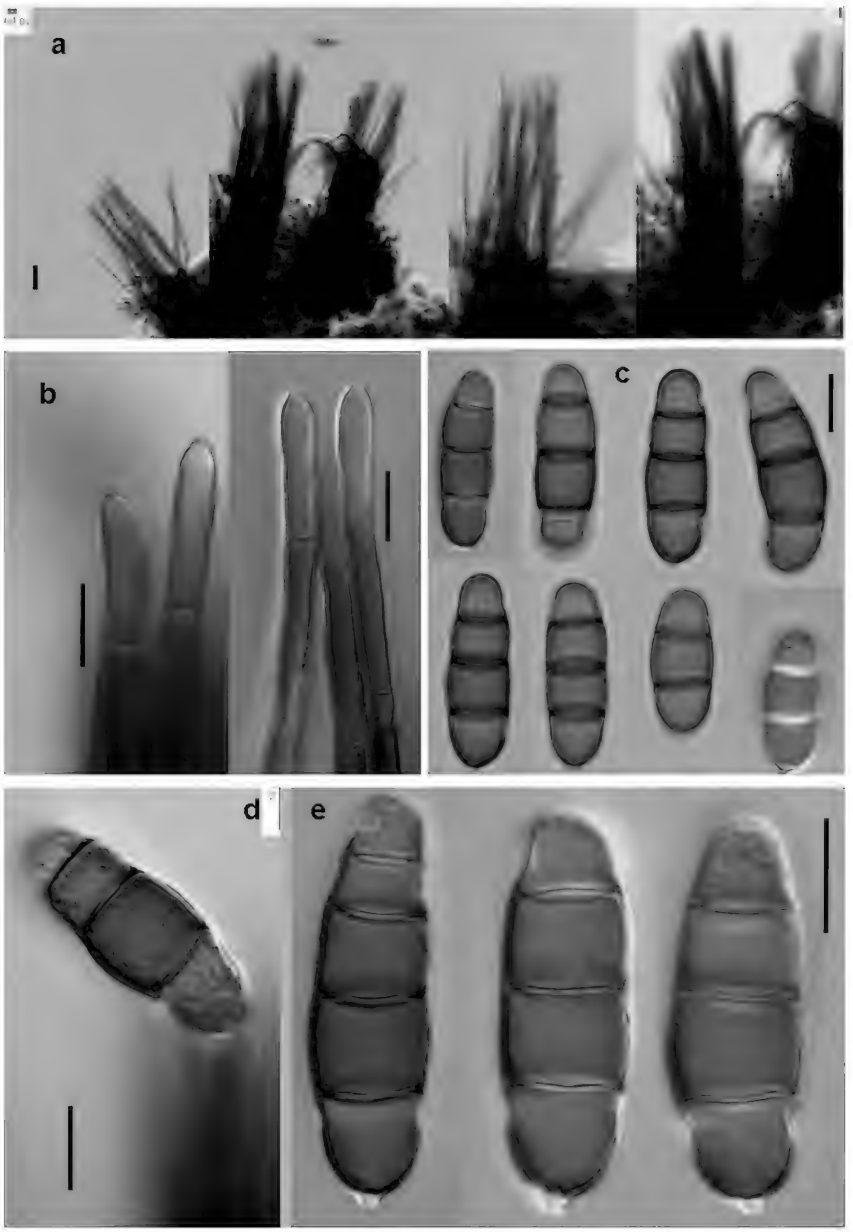

Fig. 1. *Ticosynnema carranzae* (ex holotype HAL 2454 F): a. Conidiomata. b. Conidiogenous cells. c. Attached conidium. d–e. Conidia. Bars = 20 µm (a) and 10 µm (b-e).

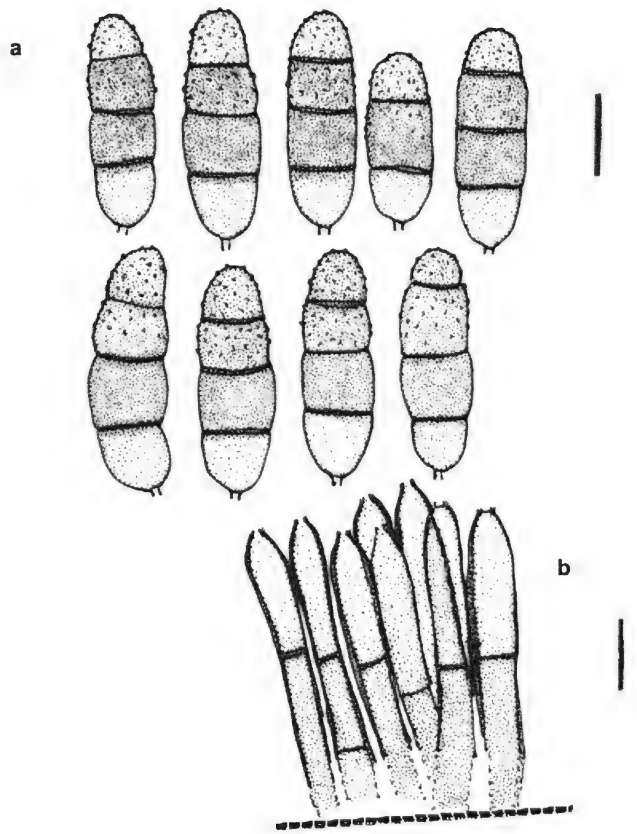

Fig. 2. Drawing of *Ticosynnema carranzae* (ex holotype HAL 2454 F): a. Conidia. b. Conidiogenous cells. Bars = 10 µm.

below, brown or pale brown above, smooth, 5–8 µm diam. Mycelium partly immersed and partly superficial. Hyphae septate, branched, smooth, pale brown, 2–4 µm diam. Conidiogenous cells monoblastic, terminal, determinate, integrated, brown to pale brown, 17–25 × 4–5 µm. Conidial secession rhexolytic. Conidia solitary, acrogenous, oblong, cylindrical, vermiform, 3–4-septate, foveate to verruculose, 35–45 × 11–12 µm, brown to dark brown, dry, with end cells usually markedly different; basal cell hemispherical, slightly narrower at the first septum, with an evident pale brown frill 1.5–2.5 µm long; apical cell somewhat obtuse. Teleomorph unknown.

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First record of *Amanita dunensis* in Italy

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Abstract — *Amanita dunensis* is recorded for the first time in Italy. It was collected in the sandy dunes of Macchiagrande Natural Reserve near Rome (Central Italy). This finding extends the geographic distribution of the species, previously recorded only in France and Spain.

Key words — in situ conservation, rare *Amanita* species, Latium littoral

Introduction

During mycological research aimed at improving knowledge of the fungal biodiversity in Macchiagrande Natural Reserve (Pecoraro & Lunghini 2003, Pecoraro et al. 2003), observations were carried out from November 1996 to January 2001 in different ecosystems included in this area.

The main object of our study was the sand dunes, one of the most interesting natural components in the research area. These dunes, which are the inner core of the coastal dune system, lie in the junction between the evergreen mediterranean maquis shrubs and sand dune environment. Their vegetation is typical of Mediterranean bush, but due to the relative abiotic parameters, species here exhibit a xeromorphic habit (e.g., *Quercus ilex* L., *Pistacia lentiscus* L., *Arbutus unedo* L., *Cistus incanus* L., *C. salviifolius* L., *Phillyrea latifolia* L., *P. angustifolia* L., *Smilax aspera* L., *Juniperus phoenicea* L., *J. oxycedrus* L.). On the other hand, truly sandy and open areas of the dune system are distinguished by psammophilous vegetation with *Pancratium maritimum* L., *Ammophila littoralis* (P. Beauv.) Rothm., and *Eryngium maritimum* L.

In this ecosystem in November 1999 we collected *Amanita dunensis*, a species remarkable due to its rarity and limited geographical distribution.

Materials & methods

The species was identified based on macro- and microscopic observations on fresh basidiomata, following the descriptions of Heim (1963) and Bon & Andary (1983). Notes on morphological characters and ecological conditions were recorded in the field, and samples were taken to the laboratory for microscopic examination. The specimens are deposited in the Herbarium D.L. of Rome (ROHB).

Results and discussion

Amanita dunensis R. Heim ex Bon & Andary, Doc. Mycol. 13(50): 13. 1983.

"*Amanita phalloides* f. *dunensis*" R. Heim, Rev. Mycol. (Paris) 28: 9. 1963, nom. inval.

Pileus 70 mm diam., applanate, not very fleshy, margin finely striate, overall color light grey with slight green tinge. Lamellae white, rather serrate, subadnate; lamellulae present. Stipe 140 × 20–25 mm, slender, subcylindrical, not bulbous, covered with sand over 2/3, whitish. Annulus present but very degraded when collected. Volva saccate, tough and resilient, whitish. Flesh whitish, with cadaverous odour, with application of sulfovanillin turning first intense violet-red, then brown.

Basidiospores 9–11.5(12) × 6.5–8 μm, amyloid, ellipsoid to elongate-ellipsoid. Basidia 50–60 × 10–12 μm, clavate, 2- or 4-spored, without clamp connections. Subhymenial trama subcellular.

Specimens examined: **ITALY. Latium**, Macchiagrande Natural Reserve, Fiumicino (Rome), 04/11/1999, leg. L. Pecoraro & D. Lunghini (ROHB D.L. 796).

Heim (1963) first described *Amanita dunensis* from France (Vendée) as *A. phalloides* f. *dunensis* (nom. inval., lacking a Latin description and type); subsequently the taxon name was validated at the rank of species by Bon & Andary (1983). This rare *Amanita* species has been reported in Spain by Castro (1998).

We collected *A. dunensis* in November 1999 for the first time in Italy, within the Macchiagrande Natural Reserve along the Latium littoral toward northern Rome.

A rare species with sabulicolous habit, *A. dunensis* is characterized by a slender aspect, non-bulbous stipe, very well-developed saccate volva, subcellular subhymenial trama, and ellipsoid to elongate-ellipsoid spores (Heim 1963, Bon & Andary 1983). It is the only known *Amanita* species with both a striate cap margin and amyloid spores. Traditionally, a striate margin in *Amanita* has been associated with inamyloid spores (Garcin 1984).

Amanita dunensis differs morphologically from *A. phalloides* (Fr.) Link in the more ellipsoid spores and distinctly striate cap margin and from *A. phalloides* var. *larroquei* F. Massart & Beauvais (Massart 1984, Neville & Poumarat 2004) by a different stipe : pileus ratio, absence of marginal pileus striations, the obvious bulbous stipe, and the pine-wood habitat.

The finding of *A. dunensis* in Italy extends its geographic distribution and emphasizes the ecological importance of the Macchiagrande Natural Reserve as an important center for conservation of rare fungal species.

Acknowledgments

We would like to thank Dr. Marco Contu, who confirmed the species identification and Prof. Giuseppe Venturella and Prof. Silvano Onofri for reviewing this article. L. Pecoraro is funded by the Estonian Science Foundation and the European Social Fund (Mobilitas Postdoctoral Research Grant MJD135).

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A new species of *Scopinella* from pampas grass in Argentina

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Abstract — A previously undescribed species of the genus *Scopinella* was found during a survey of the fungi associated with *Cortaderia selloana* (*Poaceae*) in Argentina. The ascospores are strongly asymmetrical and have the shape of a truncated pyramid with a convex quadrangular base. This peculiar ascospore shape sets this species, described here as new, apart from the others of the genus. This is the second record of *Scopinella* from South America.

Key words — ascomycete, grasses, saprophytic fungi

Introduction

Cortaderia selloana (pampas grass) is a perennial tussock-forming grass species native to Argentina, Brazil, Chile, Paraguay, and Uruguay (Astegiano et al. 1995). Its huge tussocks are very conspicuous and become attractive when they flower to produce showy feathery inflorescences. Pampas has been introduced in many regions of the world as a garden plant, dryland forage, soil amendment, and windbreaks. In some of these regions, it has become invasive (Pausas et al. 2006). The grass has become a particularly serious weed in New Zealand, where the feasibility of subjecting it to biological control is being explored (Bellgard et al. 2010).

Most records of fungal species associated with *C. selloana* are the result of studies performed in the countries where it has been introduced (Bellgard et al. 2010, Farr & Rossman 2012, McKenzie et al. 2007, Medd et al. 2003). Studies are now being conducted in Argentina to learn about the associated mycobiota present in its native distribution area.

During a survey of the fungi associated with *C. selloana* leaves in Buenos Aires province, a new species of *Scopinella* Lév. was discovered.

The genus *Scopinella* is characterized by ascomata with long necks and globose bodies, evanescent asci, and cuboid-ellipsoidal ascospores with two prominent longitudinal germ slits (Cannon & Hawksworth 1982, Stchigel et al. 2006). *Scopinella* is a small genus comprising seven species that grow on either fungi or plant substrates. It belongs to *Hypocreales*, where at present it cannot be assigned to any family (Lumbsch & Huhndorf 2010).

The shape of the ascospores of the specimen found on *C. selloana* distinguishes it from the other *Scopinella* species, so we describe it here as new.

Materials & methods

Partially senescent leaves of *Cortaderia selloana* were carefully inspected under a Wild M5A stereomicroscope. Sections bearing fruiting bodies were hand-made with a razor blade and mounted in either distilled water alone or with the addition of phloxine. Measurements were made in water. A Leica DM2000 dissecting microscope with a Leica EC3 camera was used to capture micromorphological images. For scanning electron microscopy intact ascomata were dehydrated in a 10–95% graded acetone series for fifteen minutes in each step, followed by three changes in absolute acetone. After critical point drying, the specimens were mounted on aluminum stubs, sputter coated with gold-palladium, and viewed with a LEO EVO 40 Scanning Electron Microscope (SEM). Recently collected leaves with the fungus were pressed and preserved in "Bahía Blanca Biología" Herbarium (BBB). The herbarium acronym follows Thiers (2012).

Scopinella pyramidospora R.M. Sánchez, L. Giord., F. Anderson & Bianchin., **sp. nov.** PLATE 1

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DIAGNOSIS: Differs from all other *Scopinella* species by its strongly asymmetrical, pyramidal ascospores.

TYPE: Argentina, Buenos Aires Province, Provincial Route No. 205, Km 308, (36°06′43″S 60°58′22″W): on senescent leaves of *Cortaderia selloana* (Schult. & Schult. f.) Asch. & Graebn. (*Poaceae*), 4.VII.2011, coll. FE Anderson (**HOLOTYPE**, BBB [MVB 1501]).

ETYMOLOGY: from the Greek πυραμίδος = pyramidal and σπόρος: spore.

ASCOMATA solitary to gregarious, superficial, settled on a dense mass of brown hyphae, globose to subglobose, with long necks, brown to black when dry, brown to paler brown at base when mounted in water, 106–150 µm high, 106–225 µm wide, peridial cells forming textura intricata. NECKS cylindrical, 206–313 µm long, 31–75 µm wide at base, ostiolum 19–38 µm wide, cells of neck disposed in textura porrecta. PARAPHYSES moniliform, ramified, septate, hyaline, 4–7 µm wide. ASCI 8-spored, unitunicate, clavate to globose, short-stipitate, evanescent, 13–28 × 10–16 µm. ASCOSPORES 1-celled, strongly asymmetrical, resembling a truncated pyramid with quadrangular, somewhat convex, base, rounded vertices, smooth-walled at optical level, verruculose under SEM (>7,000×) with a dark brown median band, paler at ends, germ slits occasionally present, 7.5–9.5 × 5.5–8.5 µm (x = 8.4 × 6.7, n = 60). ANAMORPH not known.

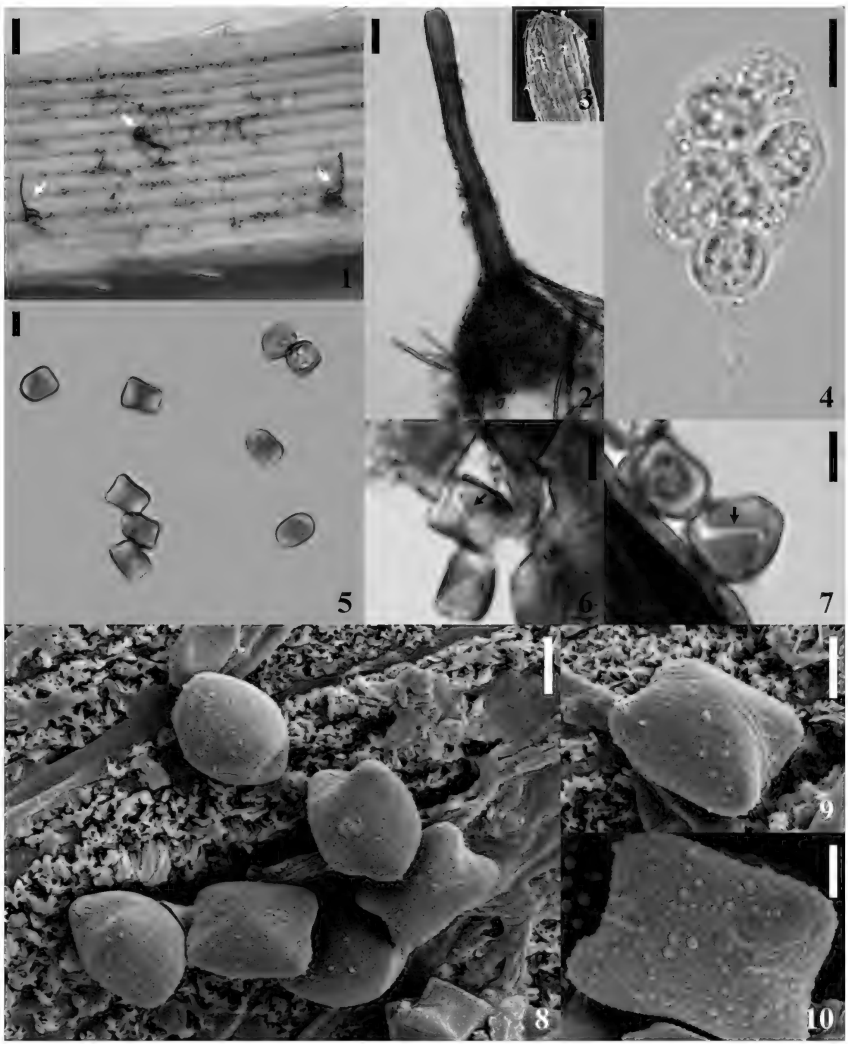

Plate 1: *Scopinella pyramidospora*: **1.** Ascomata on the substrate (arrows). **2.** Close-up of an ascoma. **3.** Apex of the ascomata neck (SEM, 4500×). **4.** Eight-spored ascus. **5.** Asymmetrical ascospores in various views. **6–7.** Ascospore germ slits (arrows). **8.** Ascospores (SEM, 7000×). **9.** Lateral view of a pyramidal ascospore (SEM, 10000×). **10.** Detail of ascospore wall (SEM, 20000×). Bars: 1 = 200 μm; 2 = 50 μm; 3 = 10 μm; 4, 6–7 = 5 μm; 5 = 7 μm; 8 = 4 μm; 9 = 3 μm; 10 = 2 μm.

Distribution— Argentina, known only from the type collection.

Comments— *Scopinella pyramidospora* differs from all other species of the genus in having strongly asymmetrical, pyramidal ascospores. Ascospore

asymmetry is best visualized by moving the microslide in three dimensions when mounted in water. Germ slits were seen only once under optical microscopy. This suggests that they are not as conspicuous and/or common as in the other species of the genus. Although the spore size falls in the range of *S. caulincola* (Fuckel) Malloch, ascospores of the latter are strongly flattened bilaterally and almost rectangular in surface view (as shown in von Arx & Müller 1954). The ascomata of *S. caulincola* have much longer necks, up to 2 mm long, with subhyaline short bristles at the apex (fide von Arx & Müller 1954). Ascomatal necks of *S. pyramidospora* are glabrous.

Discussion

Scopinella was erected by Léveillé (1847) to accommodate the single species *S. barbata* (Pers.) Lév. Saccardo (1891) added a second species, *S. pleiospora* (J. Schröt.) Sacc., originally described as *Melanospora pleiospora*. The genus was then ignored for almost a century until Hawksworth (1975) reestablished it by recognizing the synonymy between *S. barbata* and *Chaetoceratostoma hispidum* Turconi & Maffei. Hawksworth (1975) considered *Scopinella* to be monospecific and did not include Saccardo's *S. pleiospora*. Malloch (1976a,b) added three species to the genus (*S. caulincola*, *S. solani, S. sphaerophila*) and provided a key to differentiate these additions and the original *S. barbata*. Two more species were added, *S. gallicola* by Tsuneda & Hiratsuka (1981) and *S. musciformis* by Stchigel et al. (2006). No reference was made to *Scopinella pleiospora* in any of these studies. We failed to access type or authenticated material and presume the type material of *S. pleiospora* has been lost.

Scopinella species fall into two ecological groups. One comprises species growing on other fungi (*S. gallicola* and *S. sphaerophila*), and the other saprobic species, usually growing on plant substrates (*S. barbata, S. caulincola, S. musciformis*, *S. pyramidospora*, and *S. solani*). *Scopinella pleiospora* was described growing on rabbit dung. *Scopinella pyramidospora* and *S. solani* are the only two species of the genus known to occur on grasses. *Scopinella solani* was found growing on dead leaves of *Poa nevadensis* in North America (Malloch 1976a) and on overwintered inflorescences of *Brachypodium pinnatum, Dactylis glomerata*, and *Elytrigia repens* in Slovakia (Pastirčák & Pastirčáková 2007). *Scopinella solani* differs from *S. pyramidospora* in having longer ascomatal necks (300–500 µm), narrower asci (7–11.5 µm), and symmetrical and smaller ascospores (4.5–6.5 × 3.8–5.8 µm).

Scopinella species are difficult to cultivate, and the species from *Cortaderia selloana* was no exception. All attempts to isolate it on artificial media failed. Because of this, and the fact that the available material is at present very scarce, it was decided against performing any molecular work. To our knowledge the only species that have been cultivated are *S. gallicola* (Tsuneda & Hiratsuka

1981) and *S. solani* (Zhang & Blackwell 2002). Tsuneda & Hiratsuka (1982) found that for *S. gallicola* both growth and fructification were stimulated in dual culture with other fungi.

Phylogenetic studies have been performed on *S. solani*, the only species that has been sequenced. Zhang & Blackwell (2002) set *S. solani* in a distinct clade in *Hypocreales* apart from *Melanospora* in contrast with what was previously accepted (Hawksworth 1975).

Scopinella was reported in Argentina and South America for the first time with the finding of *S. solani* on bark of *Geoffroea decorticans* (Bianchinotti 1998). This is the second record of the genus from this region.

Acknowledgments

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Development and morphology of *Lysurus cruciatus* — an addition to the Indian mycobiota

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Abstract — *Lysurus cruciatus* (*Phallomycetidae*, *Phallaceae*) produces a basidiome with a long receptacle terminating in conical arms that branch initially and interconnect at their tips to support gleba on lateral surfaces. Its development from primordia to mature basidiome has been microscopically examined. Fructification begins as a small protuberance that differentiates into a series of layers and with gelatinization develops into an egg. The matured fruitbody stands up with an expanded receptacle. *Lysurus cruciatus* is a new record for the India mycobiota.

Key words — hymenium, intermediate tissue, medulla, ontogeny, peridial tissues

Introduction

Although morphology and taxonomy of phalloids have been frequently studied throughout the last century, their developmental characters have rarely been examined (e.g., Burt 1896, Fischer 1910a, Atkinson 1911, Petch 1911, Cleland & Cheel 1917, White 1944, Flegler & Hooper 1980, Sáenz et al. 1983, Zang & Peterson 1989, Abrar et al. 2007). In India, studies on *Phallaceae* associated with basidioma development are very scanty as previous reports were mainly focused on documentation (Narasimhan 1932, Ahmed 1939, Apte 2005), and there are no reports of *Lysurus* from India (Jamaluddin et al. 2004).

The tropical genus *Lysurus* is saprobic (Schaffer 1975, Lodge & Cantrell 1995) and morphologically characterized by short columnar arms (Teng 1996) attached to the apical margin of a cylindrical receptacle (Fischer 1886) that emerges from a ruptured peridium (Zang & Peterson 1989). The gleba, which is located on the lateral surfaces of receptacle arms (Miller et al. 1991, Beltrán-Tejera et al. 1998, Cortez et al. 2011), emits a fetid odor (Burk et al. 1982). *Lysurus cruciatus* is commonly known as lizard's claw stinkhorn in U.S.A. (Arora 1986).

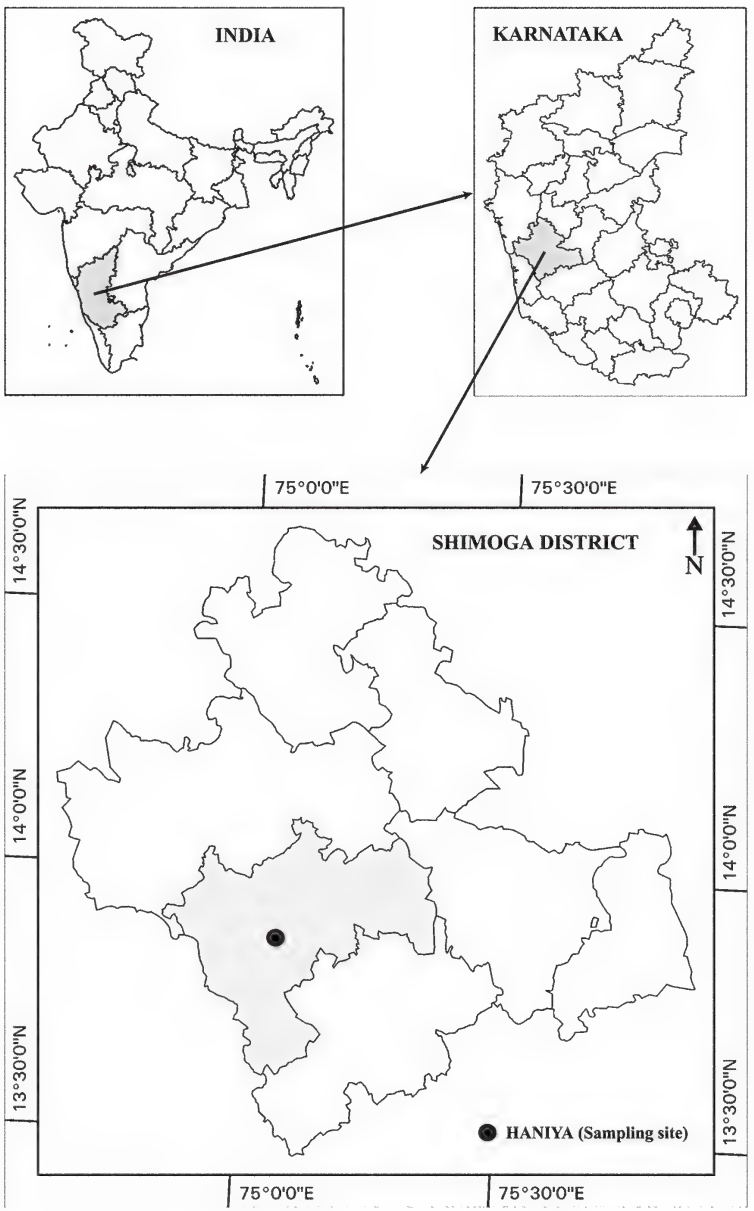

Fig. 1. *Lysurus cruciatus* sampling location in Haniya, Shimoga district, India.

In this report of a new record of *L. cruciatus* from the Indian subcontinent, we focus on the developmental stages of the fruitbody.

Materials & methods

Primordia and sequentially matured basidioma of *L. cruciatus* were collected from a site in the Western Ghats, Karnataka (Fig. 1). Macroscopic characters were described from observations of fresh material; color notations are from Kornerup & Wanscher (1981). The primordia from different stages were fixed in Pfeiffer's solution containing absolute methanol and 40% formalin (w/v) in equal proportions. All measurements were made in 3% KOH. Free hand sections of primordial stained with 1% lactophenol cotton blue and 1% phloxine were mounted on glass slides and examined under the stereo and optical microscopes. Cited collections are deposited in the Applied Botany Herbarium, Kuvempu University, Shankaraghatta, Shimoga District, Karnataka, India (KUABSAK).

Taxonomic description

Lysurus cruciatus (Lepr. & Mont.) Henn., Beibl. Hedwigia 41: 172, 1902 Figs. 2, 3a
MycoBank 100531

Immature Fruit Bodies ('eggs') 45–60 mm in diam., spherical to obovoid, white (1A1) always arising from white (1A1) mycelium cord buried under the soil. Receptacle consisting of a stipe and apical branches or arms. Stipe 85–100 × 20–25 mm, cylindrical, narrowly obconical, hollow, 3–4 mm thick, longitudinally grooved and transversely ribbed, white (1A1), composed of a pseudoparenchyma with cells of 19.2–30.5 × 17–31.6 µm, globose to subglobose. Receptacle arms 25–40 × 6–8 mm (lengths variable within the same sporome), 4–6 (usually 5), conical, constricted at the top, hollow, a deep marked groove running along the whole abaxial surface of arms, transversely wrinkled in outer surface, initially fused at their tips, then free tending to curve slightly away from the axis of the receptacle, outer concave surface smooth, white, inner convex surface transversely rugulose, with pseudoparenchyma cells of 16.5–25.6 µm diam., globose to subglobose. Gleba in the inner convex surface of the arms, brown to grayish brown (6E2-6E3), gradually deliquescing, unpleasant stinkhorn odor. Volva 35–40 × 20–25 mm, saccate, thick, white (1A1), with aerolations, composed of scattered hyphae 2.4–3.6 µm wide, stipe and volva separated by a clear jelly. Mycelial cords proliferating from the base, several white. Basidiospores 4–4.5 × 1.5–2 µm, elliptic to oblong, thin-walled, smooth, hyaline.

Ecology & Distribution: Gregarious growing in areca nut plantations in the tropical zone. Fruiting from July to October with a peak in August. Also known from America, Asia, Australia, Africa. Introduced in Europe.

Specimens examined – **India, Karnataka**, Western Ghats, Shimoga district, Haniya, 4 km from Hosanagar taluk, 13°52′58″N 75°03′27″E, alt. 742 m, S. Abrar, S. Swapna, &

M. Krishnappa, 28.VII.2007 (KUABSAK-MSH10); 21.VII.2008 (KUABSAK-MSH30); 08.X.2009 (KUABSAK-MSH102).

Developmental stages FIGS. 3B–F, 4

EGG PHASE: Initially small (0.5 mm diam.) white (1A1) swellings form at the end of mycelial strands. The 1–2 mm diam. primordia or young sporoma (YS) differentiate into a central medulla (M) and peripheral cortex (C). As differentiation continues, primary volva-gel (VG) forms from the medulla while the cortical cells fold inward (IF) four to six times. These infoldings differentiate and enlarge into lobes (L). The enlarging lobes gradually compress cortical cells to form a layer of intermediate tissue (IT) 2–4 mm in diam. The infoldings or clefts (CF) increase at the junction of intermediate tissue and medulla with the differentiation of peridial plates (PP) (4–5 mm in diam.). The external medullary region differentiates into palisade tissue (PT), comprising the primordium of the gleba (G) and develops sequentially to form enlarged clefts (ECF) (5–6 mm diam). Intermediate tissue continuously thickens along the clefts to initiate formation of receptacle arms (IRA) while the medullary tissue thickens to form the initials of the receptacle (IR) (6–8 mm diam.). The gleba enlarges (EG) (8–10 mm diam.) with the complete transformation of palisade tissue. The gleba primordium further differentiates hyphae that form the evenly arranged probasidial tips and later matures to form hymenium and trama layers. The continuous glebal development along distorted peridial plates breaks the volva-gel, which corresponds to receptacle arms (RA) (10–20 mm in diam.). The gleba gelatinization (GG) and the development of pseudoparenchymatous tissue of receptacle (R) (20–40 mm diam.) increases the size of the egg. The gleba is then reduced (RG) as grooves (GR) form outside each receptacle arm (40–45 mm diam.). Maturation proceeds with further differentiation of pseudoparenchyma in the receptacle wall and receptacle arms and with the continuous production of basidiospores from the pseudohymenial layer.

During development, the primordial cortex gives rise to the outer skin (peridium), peridial plates, and receptacle, while the volva-gel and gleba derive from the medulla. Before rupture, the mature egg differentiates into outer skin and inner volva-gel that encloses the gleba, receptacle, and receptacle arms. Complete development and differentiation of the various egg parts up through basidiospore formation occurs in 6–13 days.

After final internal development (basidiospore production), eggs become epigeous from an initial hypogeous state. Before rupture, the pre-differentiated

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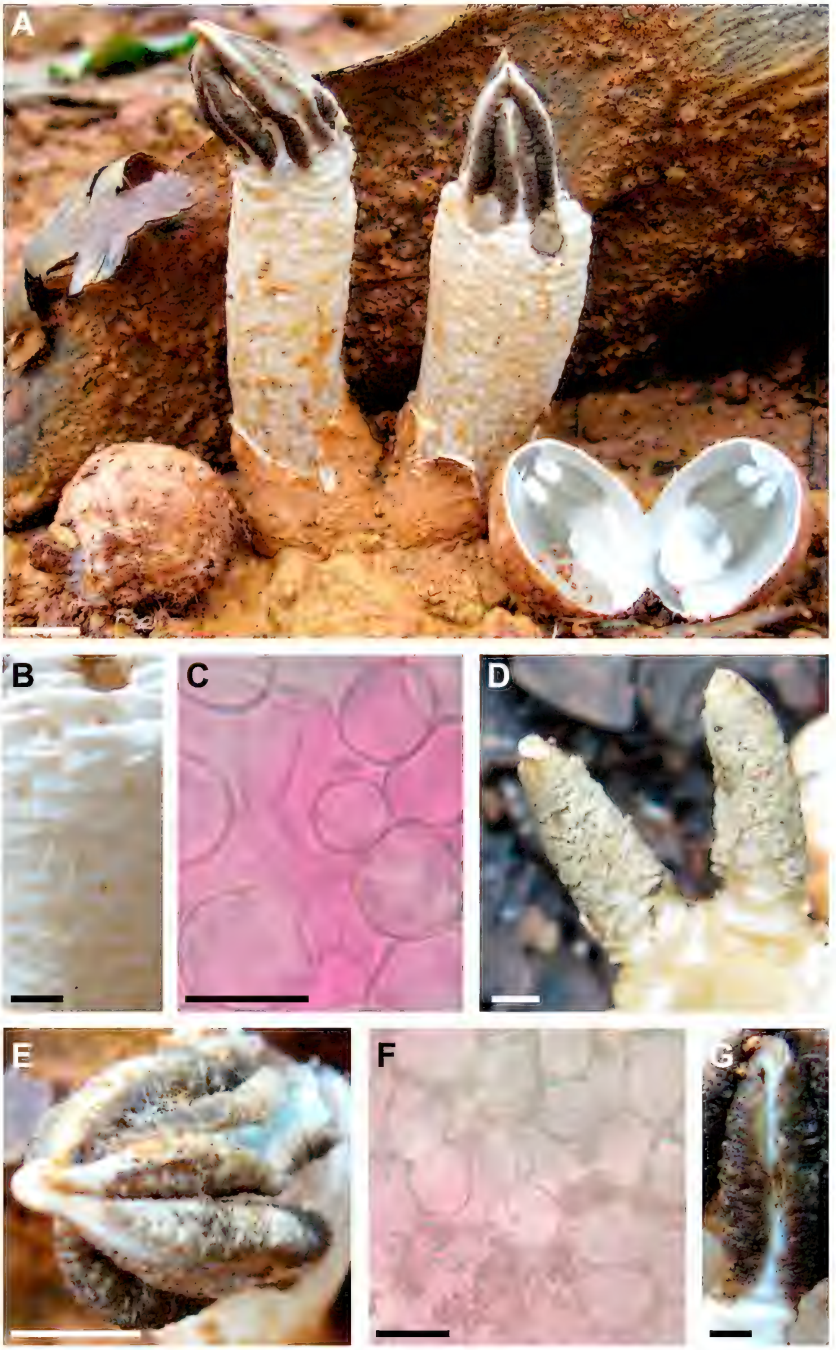
FIG. 2. *Lysurus cruciatus* (KUABSAK-MSH 30). A, Mature receptacle. B, Receptacle with transverse and longitudinal grooves. C, Pseudoparenchymatous tissue of receptacle. D, A groove underneath arm. E, Arms united at their tips. F, Pseudoparenchymatous tissue of an arm. G, An arm with external longitudinal furrow. Scale bars: A, E= 1cm; B, D = 5mm; C = 30µm; F = 20 µm; G = 3mm.


A
B
C
D
E
F
G

sporome within the egg is surrounded by a pale grayish gelatinous mucilage. A crack occurs, tearing at the apical portion (corresponding to the tip of the arms) of the egg.

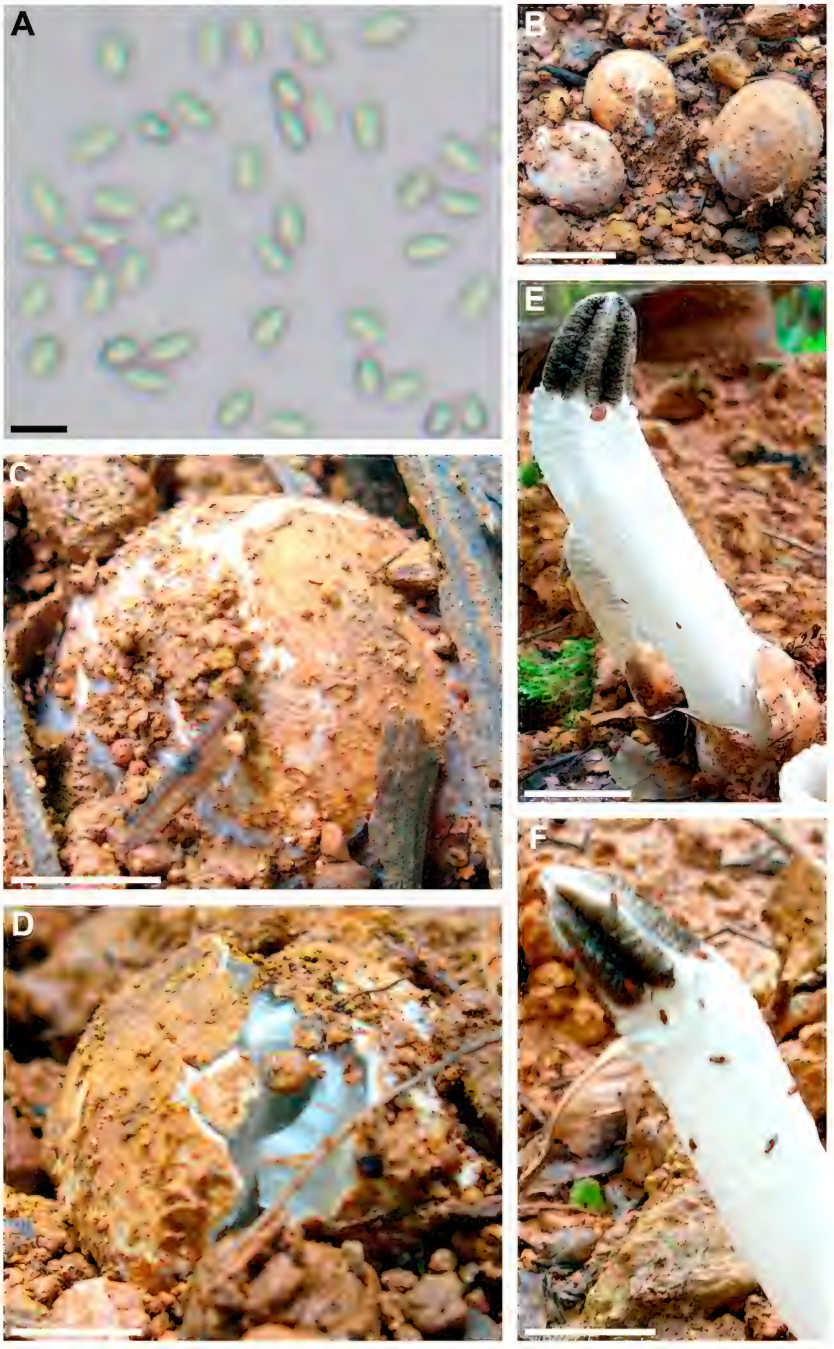
Receptacle phase: The receptacle elongates through the crack due to osmosis and cell elongation. The receptacle expands freely in 2–4 seconds with the autolysis of the gleba upon the receptacle arms (closely compressed together in the egg stage). The peridium is retained as a volva with mycelial strands at the base. The arms are set free with the moistening of the gleba, after which the gleba deliquesces and becomes fetid (Fulton 1889). Insects especially associated with carrion are attracted towards the sticky gleba. The mature phalloid basidiome, which lasts for 20–34 hrs, then autodigests and disintegrates. Under favorable environmental conditions, the dispersed basidiospores germinate to form mycelium. Development proceeds with the formation of eggs and the life cycle continues.

Discussion

Although Corda (1842) placed the genus *Lysurus* in a new family, *Lysuraceae* (as "*Lysuroideae*"), which he separated from *Clathraceae,* many 20th century mycologists treated *Lysuraceae* under *Clathraceae* (Cunningham 1944, Dring 1980, Zeller 1949). Fungal classification resulting from the Assembling the Fungal Tree of Life (AFTOL) project revealed *Phallaceae* and *Clathraceae* as separate families with *Lysuraceae* more closely related to *Phallaceae* than to *Clathraceae* (Hosaka et al. 2006). *Lysuraceae* fruitbodies resemble *Clathraceae* in having sutures dividing the gelatinous layer but differ in the long receptacles that are stipitate, longer than the arms arising from the receptacles, and with a gleba always tending to migrate to the exterior part of the arms (cf. the gleba in *Clathraceae* being attached only to the interior face of the arms). Currently, *Lysurus* is placed under *Phallaceae*, order *Phallales*, subclass *Phallomycetidae*. The phylogenetic position of *Phallales* indicates that that stinkhorns are derived from truffle-like fruitbodies (Hosaka et al. 2006, Hibbett et al. 2007).

In *Aseroe arachnoidea* E. Fisch., the peridial plates are produced radially from gleba (Fischer 1910b) whereas in *Protubera* sp., *Phallus impudicus* L., and *Ithyphallus tenuis* E. Fisch., the plates develop from the columella (Fischer 1887, Gäumann & Dodge 1928). In *Mutinus bambusinus* (Zoll.) E. Fisch., the plates develop from the apical part of the undeveloped cap (Fischer 1886) and in *Clathrus delicatus* Berk. & Broome the junction of the cortical tissue and clefts give rise to the peridial suture (Swapna et al. 2010). In *Lysurus cruciatus*, it develops from the junction of the intermediate tissue and medulla.

Fig. 3. *Lysurus cruciatus*. A, Basidiospores. B, Epigeous egg. C, D, Crack occurring at the apical portion of the egg. E, Expanded receptacle from egg. F, Insects feeding on gleba. Scale bars: A = 5μm; B, C = 1cm; D–F = 2 cm.


A
B
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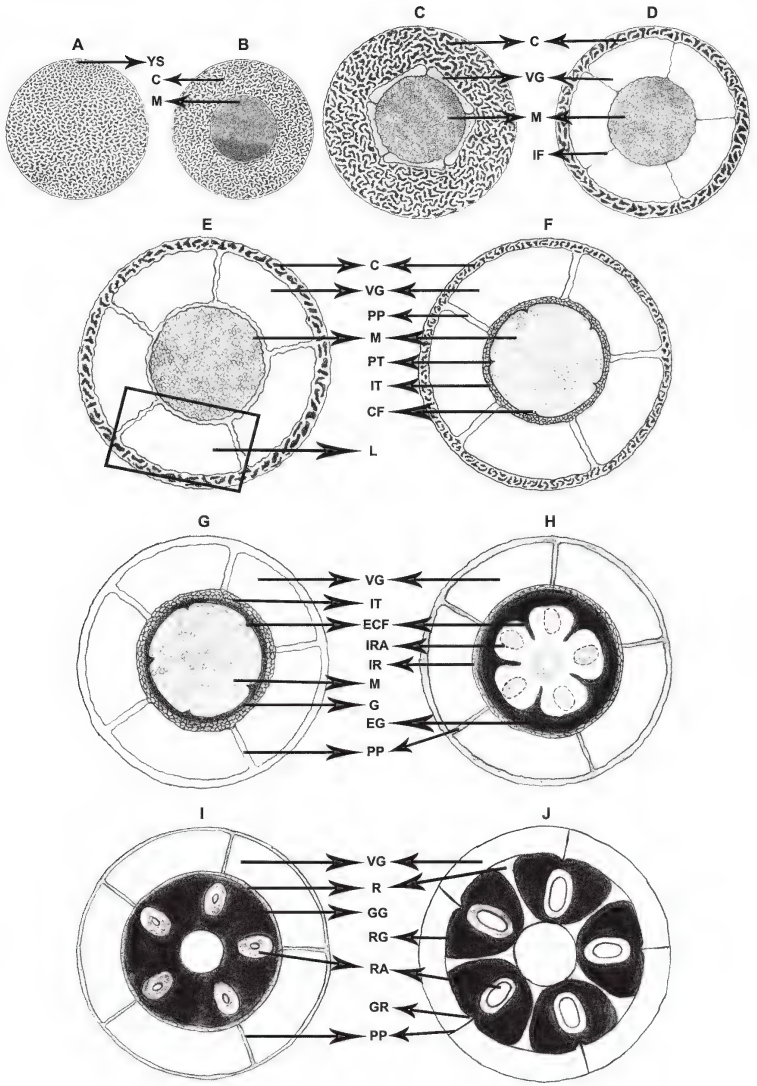
F

In *A. arachnoidea,* jelly begins to form as the stalk primordium develops (Fischer 1910b), while in *Protubera* sp., *Mutinus caninus* (Huds.) Fr., and *I. tenuis* jelly formation begins during formation of the young fruiting body (Gäumann & Dodge 1928; Fischer 1886). In *L. cruciatus* and *C. delicatus,* the jelly is formed by the differentiation of medulla (Swapna et al. 2010).

In *Simblum sphaerocephalum* Schltdl. thick strands of tissue form the central branches that run radially outwards from the center and fuse into volva-jelly at the middle of one of the receptacle meshes (Conard 1913). In *Aseroe arachnoidea* the branches are borne from the central strand (Fischer 1910b) but in *Protubera* sp., are initiated from the columella. In *M. caninus*, the cap forms from a central intertwined portion whereas in *Phallus impudicus*, the cap develops from intermediate tissue (Gäumann & Dodge 1928). In *I. tenuis* the stalk develops before forming a cap at its apex (Fischer 1886). In *C. delicatus*, the branches arise from the hyphal knots (Swapna et al. 2010) whereas in *L. cruciatus* the intermediate tissue constantly thickens to form receptacle arms.

The glebal chambers of *Aseroe rubra* Labill. develop by formation of pseudoparenchyma cells, after which trama plates are fused together and the spore mass develops horizontally around the mouth of the attached stalk (Fischer 1910b). In *M. caninus* the gleba develops during formation of the primordial plates followed by the trama and hymenium (Gäumann & Dodge 1928). In *I. tenuis* the apex of the head wrinkles to form trama wherein it extends as a dense palisade layer and bulges to form the hymenium. Later the trama assumes a gelatinous texture and sporulation initiates (Fischer 1886). In *C. delicatus* the palisade tissue transforms into gelatinized columella and the trama adheres tightly in developing receptacle, before the basidiospores form (Swapna et al. 2010). In *L. cruciatus* the external medullary region differentiates into palisade tissue constituting the glebal primordium. The gleba enlarges with differentiation of the probasidial tips and matures to form pseudohymenium, trama, and basidiospores.

According to Mycobank, six *Lysurus* species are currently accepted. *Lysurus cruciatus* and *L. gardneri* Berk. both produce white to pale cream columnar receptacle, which distinguish them from *Lysurus mokusin* (L.) Fr., characterized by well marked white to reddish angular fluted receptacle. In *L. mokusin,* the receptacle arms lie directly on the glebal columella in contrast to the arms separated with deep grooves in *L. cruciatus. Lysurus periphragmoides* (Klotzsch) Dring is unique having subglobose to ovoid clathrate network of arms with mostly pentagonal to hexagonal meshes that are at first yellow and then orange red (Dring 1980). *Lysurus pakistanicus* S.H. Iqbal et al. resembles *L. periphragmoides* in its white to yellowish hollow, narrowly obconical receptacle but differs from the latter by an irregularly oblong or globose apex formed by



FIGS. 4. Sporoma development of *Lysurus cruciatus* (KUABSAK-MCH 30). A, Primordium. B, Differentation of medulla and cortex. C, Differentiation of volva-gel. D, Cortical cells forming infoldings. E, Formation of lobes. F, Formation of intermediate tissue and peridial plates. G, Formation of palisade tissue. H, Initiation of receptacle and receptacle arms. I, Development and gelatinization of gleba. J, Formation of grooves and reduction of gleba. ABBREVIATIONS: C = Cortex, CF = Cleft, ECF = Enlarged cleft, EG = Enlarged gleba, G = Gleba, GG = Gelatinization of gleba, GR = Groove, IF = Infolding, IR = Initiation of receptacle, IRA = Initial of receptacle arm, IT = Intermediate tissue, L = Lobe, M = Medulla, PP = Peridial plate, PT = Palisade tissue, R = Receptacle, RA = Receptacle arm, RG = Reduction of glebal mass, VG = Volva-gel, YS = Young sporoma.

a clathrate network of anastomosing arms, irregular to cylindrical with up to 10 meshes that is rosy to orange pink at maturity; in *L. pakistanicus* the gleba fills the entire interior of the arms and extends outwards in the meshes (Iqbal et al. 2006). *Lysurus corallocephalus* Welw. & Curr. is entirely different, with erect appendages that are usually dark or flushed pink above and white to buff or yellowish below and which have simple or forked with slender intersections.

Lysurus cruciatus differs from the other known *Lysurus* species by the uniquely transversely rugulose glebiferous surface. The glebiferous region is villose and lamellate in *L. gardneri,* slightly keeled on both outer and inner surfaces in *L. pakistanicus* and *L. periphragmoides,* and corrugated on the sides and flat on the inner surface *L. periphragmoides* is (Dring 1980, Iqbal et al. 2006). The gleba is on the lateral surface of the arms in *L. mokusin* and distributed on the exterior of polygonal meshes in *L. corallocephalus* (Dring 1980).

In *L. gardneri* the stalk divides at the apex and does not bear any glebiferous layer for 2–4 mm from the base of the arm. Each arm is well marked and borne on its own stalk with a narrow zone, with the sterile base appearing as a furrow down the outer face (Petch 1911). The diameter of the arms at 3 mm above the stalk region increases to 6 mm in the glebiferous region. The gleba of *L. cruciatus* runs along the groove in the intersection without any sterile base. The diameter of the arms is almost the same through its length. The glebal content is comparatively less in *L. cruciatus* compared to *L. gardneri,* which is more concentrated in the center of the arms with a bulging appearance and reduced towards the tips.

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New *Anthracoidea, Tilletia,* and *Ustilago* records for Turkey

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Abstract — Three smut taxa, *Anthracoidea angulata* on *Carex hirta*, *Tilletia lolii* on *Lolium perenne*, and *Ustilago serpens* on *Elymus elongatus*, are reported for the first time from Turkey. The morphological and microscopical features of these fungi are described based on the collected materials.

Key words — Anatolia, Ardahan, Kars, *Ustilaginales*

Introduction

The smut fungi (*Ustilaginales*) are parasites of cereals and other cultivated and natural plants, comprising more than 1400 species in approximately 70 genera (Vanky 1994).

In the last decade, reports of new records of smut fungi have greatly increased in Turkey, and 55 smut species in 9 genera have been determined for the country (Bahcecioglu & Yildiz 2005; Bahcecioglu et al. 2006; Bremer et. al. 1952; Kirbag 2003; Kabaktepe & Bahcecioglu 2006; Petrak 1953; Sahin & Tamer 1998; Sert et. al. 2004; Sert 2009). Further smut research is required, as this number is far less than the total number of potential host plants in Turkey.

This paper presents smut species collected from Kars and Ardahan provinces in northeast Turkey.

Materials & methods

Specimens were collected during the field studies in 2006–09 from Kars and Ardahan Provinces in Turkey. The host specimens were prepared according to established herbarium techniques. Spores were scraped from dried host specimens and mounted in lactophenol. The preparations were examined with an Olympus CX31 light microscope and micrographs taken with a Olympus E–330 camera. Analysis LS Starter software was used to measure at least 30 spores for each spore state. Host names follow Davis (1965–85)

and Davis et al. (1988). Specimens are deposited in the Inonu University Herbarium (INU), Turkey. The smut species were identified using relevant literature (Mordue & Ainsworth 1984; Vanky 1994) concerning smut fungi in Europe.

Taxonomy

Anthracoidea angulata (Syd.) Boidol & Poelt, Ber. bayer. bot. Ges. 36: 23 (1963)

Sori in inflorescence. Spores, angular dark brown, 12–20 × 15–28 µm wall irregularly thickened, 1.5–5 µm, verruculose.

A new smut species for Turkey, *A. angulata* is also known on *Carex hirta* from Europe and on *C. melanostachya* Willd. from Asia.

Specimens examined – On *Carex hirta* L. (*Cyperaceae*). **TURKEY**: **Kars**, 10 km from Sarikamis to Horosan, 2010 m, 22.09.2006, Sanli Kabaktepe 4653 (INU 8549); **Ardahan**, Yalnizcam mountains, Bilbilan plateau, 2400 m, 18.07.2007, Sanli Kabaktepe 5422 (INU 8550).

Tilletia lolii Auersw. ex G. Winter, Rabenh. Krypt.-Fl. Ed. 2, 1(1): 109 (1881)

Sori in ovaries, covered by epidermis. Spores, globose to subglobose, 16–24 µm, light brown, wall reticulate, reticulations 2–5 µm wide 1.5–4 µm deep. Sterile cells, few, globose, subglobose, 10–20 µm, hyaline, wall 1–2 µm.

New for Turkey, *T. lolii* is also known from Asia, Europe, and New Zealand.

Specimens examined – On *Lolium perenne* L. (*Poaceae*). **TURKEY**: **Ardahan**, Posof, 2–4 km east of Kursuncavus village, 1720 m, 28.08.2008, Sanli Kabaktepe 6544 (INU 8552).

Ustilago serpens (P. Karst.) B. Lindeb., Symb. bot. upsal. 16(2): 133 (1959)

Sori in leaves, covered with epidermis. Spores globose, ellipsoidal 11–20 µm, dark brown, wall 0.5–1 µm, verruculose.

New for Turkey, *U. serpens* is also known from Europe, Asia, North America, and New Zealand.

Specimen examined – On *Elymus elongatus* (Host) Runemark (*Poaceae*). **TURKEY**: **Kars**, 13 km from Kagizman to Kars, 1350 m, 11.06.2007, Sanli Kabaktepe 4918 (INU 8551).

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A new species of *Coccomyces* (*Rhytismatales, Ascomycota*) on *Ilex elmerrilliana*

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Abstract —*Coccomyces ilicis* sp. nov. is described, illustrated, and compared with similar taxa. This fungus was collected from fallen leaves of *Ilex elmerrilliana* in Mt Huangshan of Anhui Province, China. It differs from the closest relatives, *C. noosanus* and *C. limitatus*, by its much wider asci and the absence of an excipulum and epithecium. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — *Rhytismataceae*, taxonomy, *Aquifoliaceae*

Introduction

Coccomyces De Not. is a large genus of *Rhytismataceae*, for which 116 species were recorded by Kirk et al. (2008) plus a few recently added. Thus far 25 *Coccomyces* species have been reported for China (Jia et al. 2012; Zheng et al. 2012). Teng (1934) published the first records for China, *C. dentatus* (J.C. Schmidt) Sacc. and *C. delta* (Kunze) Sacc. Most subsequent Chinese reports have been made by Lin and Hou (Lin et al. 1994, 1999, 2000a, b, c, 2001; Hou et al. 2006; Hou & Piepenbring 2007; Jia et al. 2012; Zheng et al. 2012). In this paper, we describe another new *Coccomyces* species from China on fallen leaves of *Ilex elmerrilliana.*

Materials & methods

Mature fruit bodies were selected from collected specimens. Macroscopic appearance was described from dried reference collection material under a dissecting microscope at 10–50× magnification. After rehydration in water, fruitbodies were cut using a freezing microtome into 8–15 µm thick vertical sections, which were mounted in lactic acid or cotton blue for observing the outlines of ascomata and conidiomata. Gelatinous sheaths surrounding ascospores and paraphyses were examined in water or 0.1% (w/v) cotton

blue in lactic acid. The colours of diversified structures and ascospore contents were observed in water. Measurements and drawings were made using materials mounted in 5% KOH and from ca 30 asci, ascospores, and paraphyses for each specimen.

Taxonomy

Coccomyces ilicis S.J. Wang & Y.R. Lin, **sp. nov.** FIGS 1–6

MYCOBANK MB 800353

Differs from *C. noosanus* and *C. limitatus* by the absence of an excipulum and epithecium, and by having much wider asci.

TYPE: China, Anhui, Mt Huangshang, Zuishi, alt. ca 700 m, on leaves of *Ilex elmerrilliana* S.Y. Hu (*Aquifoliaceae*), 11 September 2004, S.J. Wang & Y.R. Lin 1878 (**Holotype**, AAUF 67986).

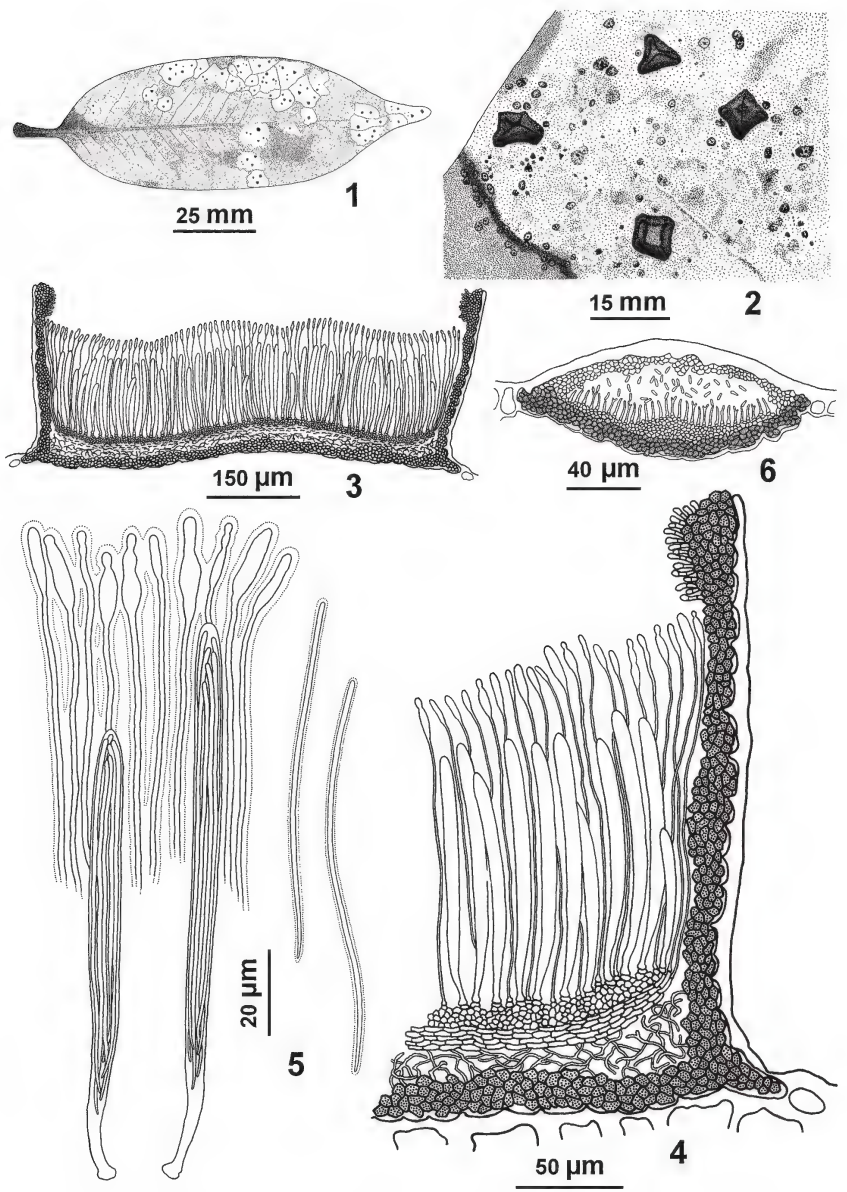
ETYMOLOGY: The specific epithet refers to the host genus.

COLONIES on both sides of leaves, forming irregular, yellowish-white paler spots.

ZONE LINES frequent, grey-brown or black, thin, entirely or partly surrounding the paler spots.

CONIDIOMATA in similar positions to ascomata on the substratum, scattered or crowded, sometimes coalescent, mostly on both sides of the zone lines. In surface view, conidiomata disparate in dimensions, 45–180 µm diam., rotund, black-brown in the centre and the perimeter line of the conidioma, yellowish-brown to grey-black elsewhere, somewhat raising the leaf surface, discharging spores through 1 to 2 apical ostioles. In vertical section, conidiomata intraepidermal, double lens-shaped. UPPER WALL 6–9 µm thick, yellowish-brown but dark brown near the ostiole(s), consisting of disparately sized angular cells. BASAL WALL extremely well developed, 12–22 µm thick, black-brown, consisting of 3–4 layers of thick-walled angular cells ca 3 µm diam. SUBCONIDIOGENOUS LAYER 5–10(–22) µm thick, composed of nearly colourless, thin-walled angular cells. CONIDIOGENOUS CELLS 10–15 × 2–3 µm, cylindrical, slightly tapering towards the apex, holoblastic sympodially proliferating. CONIDIA 3–5 × 1–1.5 µm, cylindrical or subclavate, hyaline, aseptate.

ASCOMATA developing on both sides of leaves, scattered, rarely 2 to 3 coalescent, in irregular, yellowish-white bleached spots. In surface view, ascomata 600–1250 µm diam., triangular or quadrangular, black-brown to black, shiny, edge defined, moderately raising the substrate surface, with an obvious performed dehiscence mechanism, opening by 3–4 radial splits nearly extend to the edge of ascoma, to expose waxy-yellow hymenium. Lips absent. In median vertical section, ascomata intraepidermal. COVERING STROMA 25–35 µm thick near the opening, slightly thinner towards the edge, connecting to the basal stroma, comprised of textura angularis-globulosa with dark brown, thick-walled cells 4–6 µm diam. Few periphysoids near opening slit. BASAL STROMA



Figs 1–6. *Coccomyces ilicis* on *Ilex elmerrilliana*. 1. A leaf bearing fruitbodies. 2. Conidiomata and ascomata observed under a dissecting microscope. 3. Ascoma in median vertical section. 4. Portion of ascoma in median vertical section. 5. Paraphyses, asci and ascospores. 6. Conidioma in vertical section.

20–25 µm thick, composed of textura angularis-globulosa with 2–3(–4) layers of black-brown, thick-walled cells 5–8 µm diam. Internal matrix stroma 14–25 µm thick, consisting of hyaline, gluey textura intricata. Excipulum absent. Subhymenium 15–22 µm thick, comprised of hyaline textura angularis and porrecta. Paraphyses 130–160 × 1–1.2 µm, filiform, septate, not branched, increasing to 3.5–5 µm at clavate, fusoid or calabash-like apex, covered with a ca 0.5 µm thick gelatinous matrix but not forming an epithecium. Asci ripening sequentially, 95–130 × 5–7 µm, clavate, apex rounded or subacute, short-stalked, thin-walled, J–, 8-spored. Ascospores arranged fasciculately, tapering towards the base, 65–95 × 1–1.2 µm, filiform, hyaline, aseptate, covered by a gelatinous sheath ca 0.5 µm thick.

Ecology & distribution: Producing conidiomata and ascomata on fallen leaves of *Ilex elmerrilliana*. Known only from the type locality, Anhui, China.

Comments —*Coccomyces* is widely distributed globally and associated with a wide spectrum of plants in 61 families, particularly *Ericaceae*, *Fagaceae*, and *Lauraceae* (Sherwood 1980). Of 24 species of *Rhytismatales* reported to associate with *Aquifoliaceae*, only two belong in *Coccomyces: C. yerbae* Speg. and *C. radiatus* Sherwood (Farr & Rossman 2012). They, however, differ morphologically from *C. ilicis* (Sherwood 1980; Johnston 1994).

Coccomyces noosanus P.R. Johnst. resembles *C. ilicis* macroscopically and microscopically but differs in having a well-developed, compact periphysoid layer, much narrower asci (4.5–5.5 µm), wider ascospores (1.5 µm), and the presence of an excipulum and epithecium (Johnston 2000). *Coccomyces limitatus* (Berk. & M.A. Curtis) Sacc. is also close to *C. ilicis*, but has smaller ascomata (500–1000 µm diam.), a paraphysis-like excipulum, an obvious epithecium, and narrower asci (4.5–5.5 µm) (Johnston 2000; Lin et al. 2000c).

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New Asian records and morphological variation in *Fibrodontia brevidens* (*Basidiomycota*)

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Abstract — *Fibrodontia brevidens* (*Trechisporales*) is reported for the first time from Taiwan, mainland China, and Vietnam. Asian specimens are compared morphologically with collections from Venezuela. Morphological variation in the species is discussed. Most variable features within the specimens are encrustation of projecting skeletal-like hyphae (from totally naked to richly encrusted) and spore size. Nevertheless, the single morphospecies concept of *F. brevidens* is retained. Basidioma photographs, microscopical drawings, and scanning electron microscopic photographs of crystalline deposits on skeletal-like hyphae of this species are provided.

Key words — corticioid, *Hyphodontia*, SEM, taxonomy

Introduction

Fibrodontia brevidens (Pat.) Hjortstam & Ryvarden (*Hydnodontaceae*, *Trechisporales*) is a presumably not rare but little collected pantropical corticioid fungus. It has been reported previously from Africa (Rwanda; Ryvarden 1978), Sunda Archipelago (Brunei; Hjortstam et al. 1998), Caribbean Archipelago (Guadeloupe; Nakasone 2003), and South America (Ecuador – Langer 1994; Venezuela – Hjortstam et al. 2005; Brazil – Hjortstam & Ryvarden 2007, S.P. Gorjón, pers. comm.; Uruguay – Martínez & Nakasone 2010). Although there have been numerous studies on the corticioid fungi of mainland China and Taiwan summarized in two recent checklists (Wu 2010, Dai 2011), *F. brevidens* has not been reported earlier for either country.

Morphological descriptions of *F. brevidens* were published by Ryvarden (1978; as *Hyphodontia africana* Ryvarden), Langer (1994; as *Hyphodontia brevidens* (Pat.) Ryvarden), and Nakasone (2003; type of *Acia sericea* Pat.).

The most prominent feature of this species (and the genus in general) are the numerous long parallel moderately flexuous thick-walled hyphae (usually yellowish in mass) that constitute the core of hymenophoral aculei and project at their apices. They have been referred to variously as thick-walled hyphae (Ryvarden 1978), skeletal hyphae (Eriksson et al. 1981, Jung 1987), tubular tramacystidia (Langer 1994), tramal cystidia or pseudocystidia (Nakasone 2003), or pseudoskeletal hyphae (Bernicchia & Gorjón 2010). Similarly, the *Fibrodontia* hyphal system has been described as semi-dimitic (Ryvarden 1978), dimitic (Eriksson et al. 1981), or pseudodimitic (Langer 1994, Bernicchia & Gorjón 2010). An argument for dimiticism is the presence of skeletal hyphae in sterile hyphal textures of the type species, *F. gossypina* Parmasto (Eriksson et al. 1981, Fig. 542). We use the term "skeletal-like hyphae" to refer to these structures in the present paper.

Materials & methods

The reference specimens, collected during 1991–2000 in Asia and South America, are stored in TNM, O, and MSK herbaria (acronyms according to Index Herbariorum, http://sweetgum.nybg.org/ih/). Microscopic structures were measured in material mounted in 3% KOH water solution, and the spore quotient (spore length/width ratio) excludes the apiculus. Encrustations were studied in Melzer's reagent and/or distilled water. The hymenial surface was photographed with Nikon Coolpix P6000 digital camera, while encrusting crystals were photographed with a Hitachi SU 1510 scanning electron microscope.

Results & discussion

Critical study of the collections stored in TNM confirms *Fibrodontia brevidens* as a new record for Taiwan, mainland China, and Vietnam. Taiwanese records are based on two localities, one in central Taiwan and the other on Orchid Island in the Philippine Sea near the southeast Taiwan coast. Two mainland China records represent one site in central Sichuan Province and a second in the extreme south of Yunnan Province. The Vietnamese localities lie in the northern part of the country in the mountains around Ha Noi. The habitats correspond to tropical (from near sea level) and warm subtropical vegetation (up to 1600 m a.s.l.).

Langer (1994) regarded the crystals on skeletal-like hyphae as a common feature in *Fibrodontia*. However, only one (TNM F8714 from Orchid Island) of eight Asian specimens examined had skeletal-like hyphae richly encrusted by characteristic flattened crystals and visible in KOH. To resolve the taxonomic position of specimens with naked hyphae, we compared their general morphology with *F. brevidens* specimens from Venezuela collected by L. Ryvarden.

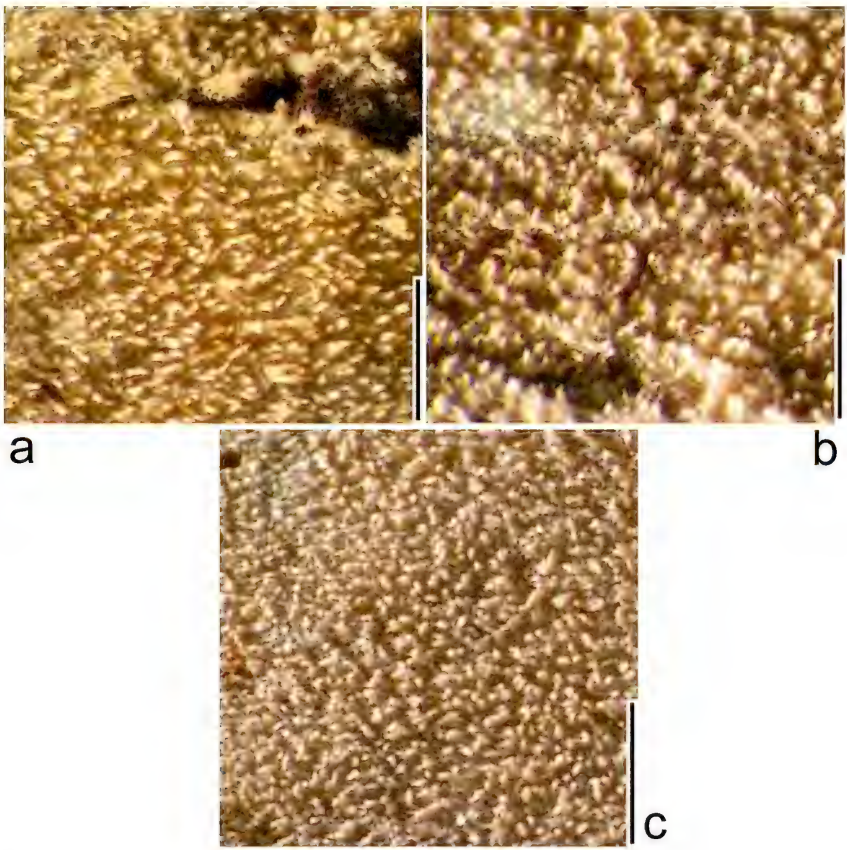

Fig. 1. *Fibrodontia brevidens*. Details of basidiomata: a, TNM F8714 (Orchid Island, Taiwan); b, TNM F24714 (central Taiwan); c, TNM F9008 (Vietnam). Scale bars = 1 mm.

Hymenophore color has been said to vary in *F. brevidens* from white or cream (Langer 1994) to ochraceous (Hjortstam et al. 1998) and greyish orange (Nakasone 2003). However, the Asian and South American specimens we examined were yellowish with olive tinges and not especially variable in color. The hymenophore consists of 50–150 µm tall aculei, which also were not too variable in density and shape (Fig. 1).

The encrustations on skeletal-like hyphae in Asian specimen TNM F8714 resemble those in both Venezuelan specimens (O 37539, O 40434; Fig. 2e, f, h, i). The other Asian specimens have totally naked to unclearly or poorly encrusted skeletal-like hyphae where the crystals were not tablet-shaped and

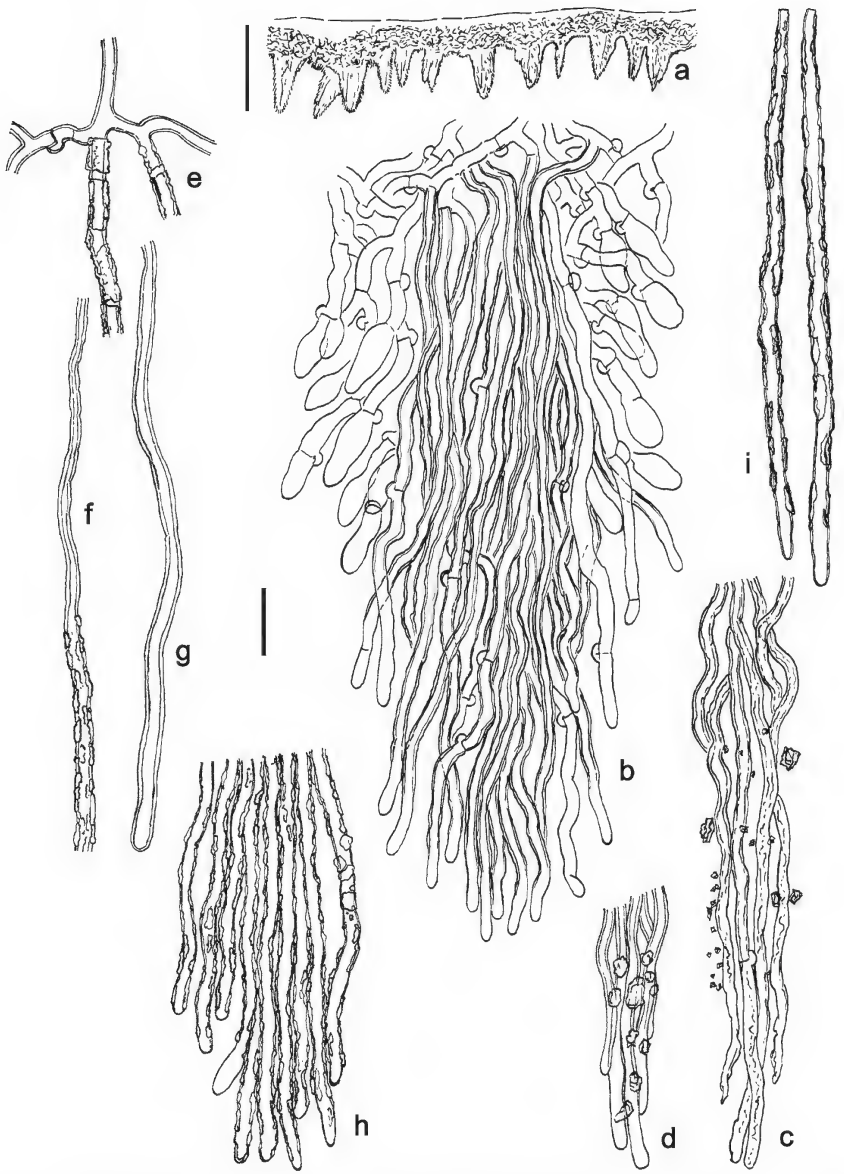

Fig. 2. *Fibrodontia brevidens*. Microscopical elements (TNM F24714): a, vertical section through basidioma; b, vertical section through an aculeus (in KOH solution); c, part of aculeal core (in water). TNM F4188: d, upper parts of skeletal-like hyphae (in water). TNM F8714, skeletal-like hyphae: e, f, in lower part; g, naked one; h, collected in emerging cluster. O 37529: i, skeletal-like hyphae. Scale bars: a = 0.2 mm; b–i = 10 μm.

often only observed in Melzer's reagent or water preparations (Fig. 2b–d). The "encrusted" specimens varied from seldom completely naked (Fig. 2g) to richly encrusted skeletal-like hyphae. Such variability within a single specimen is also inferred from the published descriptions and illustrations of *F. brevidens* (Ryvarden 1978, Langer 1994 – specimen Rammeloo 3929) and *F. gossypina* (Eriksson et al. 1981). Nakasone (2003) provided no data on encrustations on tramacystidia in *Hyphodontia brevidens*. We thus attribute the degree of encrustation to intraspecific variation, partly explained by the developmental stage of each cluster of skeletal-like hyphae.

The shape of the crystals on skeletal-like hyphae in *Fibrodontia* is characterized as tablet-like and partly cuff-like (Langer 1994). However, as their shape is poorly interpreted using light microscopy alone, we compared the two *F. brevidens* skeletal-like hyphal morphotypes (clearly encrusted vs. naked) under the scanning electron microscope. Our SEMs demonstrated that the number and shape of crystals depend on the age of sample examined. The "encrusted" morphotype produced younger aculei with subacicular loosely attached tablet-shaped crystals (Fig. 3a) and older aculei encrusted by numerous tablet-shaped, slate-shaped, and small-sized prismatic crystals (Fig. 3b,c); here no circularly girdling crystals were observed. In the 'naked' *F. brevidens* morphotype, we observed absolutely no encrustations in younger basidioma patches (Fig. 3d), while older aculei contained some skeletal-like hyphae very slightly roughened from scarce minute crystals (Fig. 3e); scattered and loosely attached semi-tablet-shaped crystals were also detected (Fig. 3f).

Published descriptions and our examinations indicate a notable spore size variation within *F. brevidens*. A Venezuelan specimen (O 37539) produced the smallest spores (3.5–4 × 2.5–3.5 µm), and a specimen from Sichuan (TNM F12152; Fig. 5) produced the largest (4.5–5.2 × (3–)3.5–4 µm). Previously published spore sizes vary from 3.5–4 µm in diam (Ryvarden 1978) to 4.5–5(–5.5) × 3.5–4.5 µm (Nakasone 2003).

The large-spored samples from Sichuan and central Taiwan were also distinguished by narrower, more flexuous, loosely arranged skeletal-like hyphae. A large-spored (3.7–4.5(–5) × 3.2–3.5 µm) sample from Taiwan (TNM F24714) had capitate hyphal ends in the subiculum similar to those in *Hyphodontia* (*Xylodon*) species, a character not previously noted for *Fibrodontia* (Fig. 4b). Between collections, spore shape (predominantly broadly ellipsoid and ovoid in *F. brevidens*) was less variable than spore size. Spore quotients within a studied specimen varied from 1 to 1.4; TNM F8714 contained some dorsoventrally flattened spores (ellipsoid in lateral view), with a length : width ratio of 1.6.

Basidial morphology in *Fibrodontia brevidens* differs from that in *Hyphodontia* s. l., which we consider an additional argument for placing *Fibrodontia* in the trechisporoid clade. Basidia in *F. brevidens* are initially

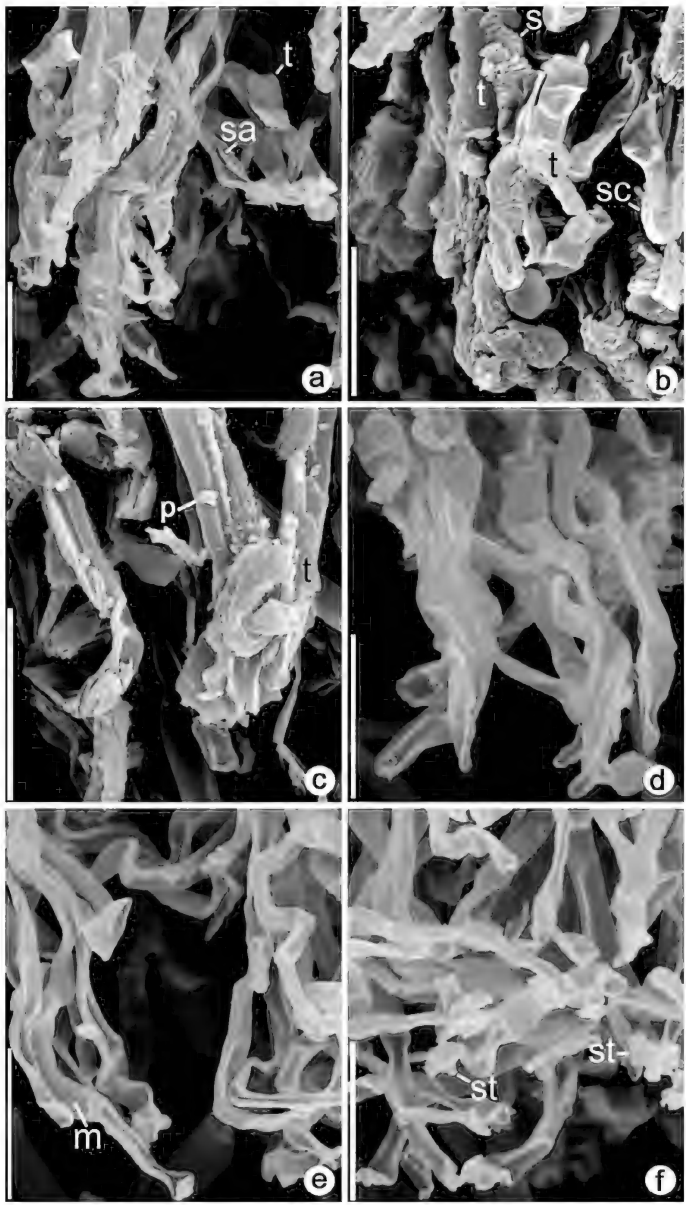

FIG. 3. Scanning electron microscopic images of projecting skeletal-like hyphae and their encrustations in *Fibrodontia brevidens*. TNM F8714: from younger (a) and older (b, c) basidioma patches. TNM F24714: from younger (d) and older (e, f) basidioma patches. The main crystal shapes are marked: m – minute, powder-like; p – prismatic, sa – semi-acicular; s – slate-like; sc – semi-cuff-shaped; st – semi-tablet-shaped; t – tablet-shaped. Scale bars = 10 μm.

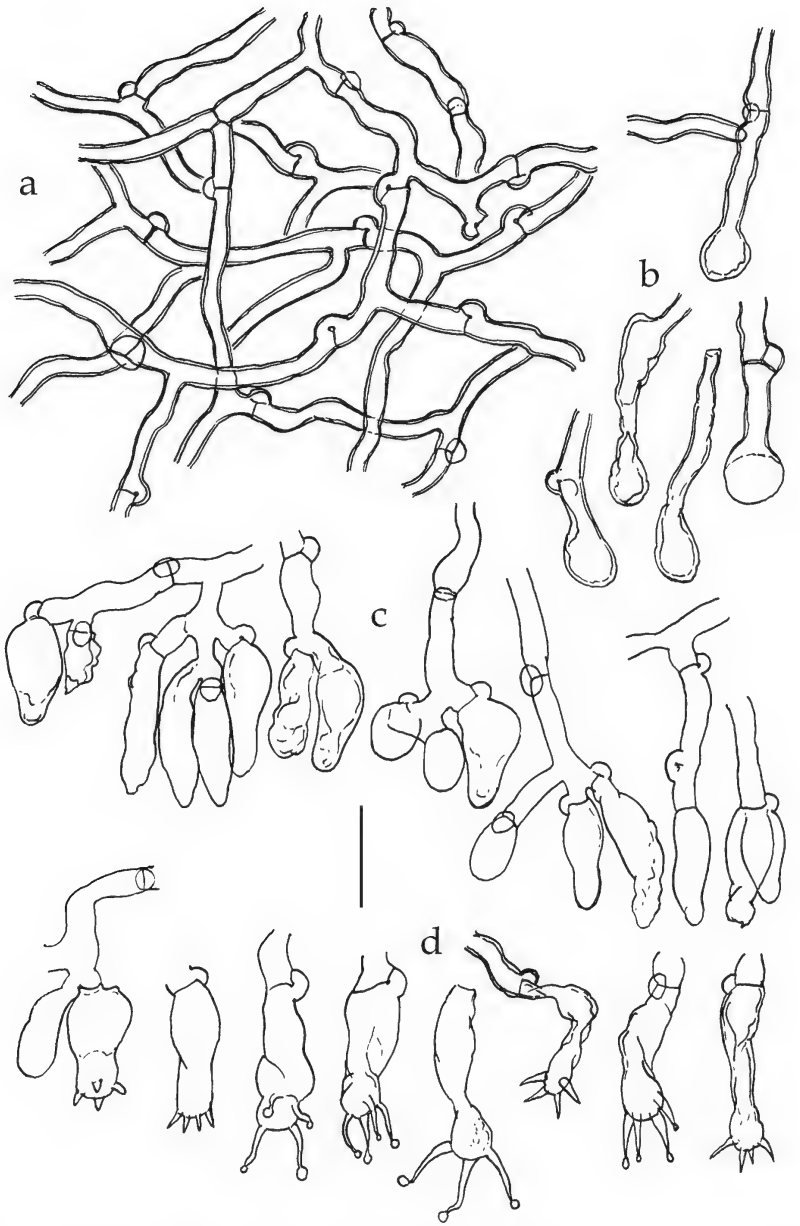

Fig. 4. *Fibrodontia brevidens*. Microscopical elements (TNM F24714): a, subicular hyphae; b, capitate hyphal ends from subiculum; c, basidioles and subhymenial hyphae; d, basidia. Scale bar = 10 μm.

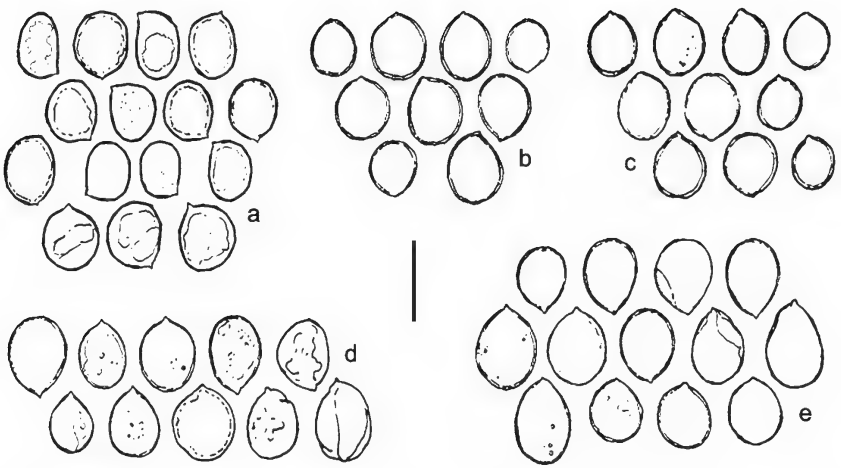

Fig. 5. *Fibrodontia brevidens*. Basidiospore variation: a, TNM F8714 (Taiwan, Orchid Island); b, TNM F4188 (China, Yunnan); c, O 37539 (Venezuela); d, TNM F24714 (central Taiwan); e, TNM F12152 (China, Sichuan). Scale bar = 5 μm.

ovoid, becoming urniform and basally swollen, then more or less utriform with somewhat wavy divaricate sterigmata, and ultimately shrinking and distorted (Fig. 4d).

Except for the encrustations on skeletal-like hyphae and smaller spores (Fig. 5c), we found no significant differences between South American and Asian specimens. From our own observations on variation in *F. brevidens* as well as morphological data by other authors, we regard all studied material as one morphospecies.

Specimens examined — **CHINA: Sichuan**, Chingchenghoushan (Qing Cheng Hou Shan), Feichuankou, 900 m a.s.l., on naked dead wood, S.H. Wu & S.C. Wu, 13.X.2000, Wu 0010-113 (TNM-F 12152); **Yunnan**, Shishuangbanna (Xishuangbanna), Nabanho, 600 m a.s.l., on bamboo stem, coll. S.H. Wu & J.Y. Tseng, 16.VIII.1995, Wu 9508-282 (TNM F4188); on bamboo stem, coll. S.H. Wu & J.Y. Tseng, 16.VIII.1995, Wu 9508-297 (TNM F4203). **TAIWAN: Nantou County**, Luku Hsiang, Sunlinksea, 1600 m a.s.l., on decorticated branch of angiosperm species, coll. S.H. Wu, 11.X.1991, Wu 911011-16 (TNM F24714; dup. in MSK); **Taitung County**, Orchid Island, Yunghsing Farm, on dead branch of angiosperm species, coll. S.H. Wu & J.Y. Tseng, 30.IV.1997, Wu 9704-179 (TNM F8714). **VENEZUELA: Estado Miranda**, Sartenejas, Univ. Simon Bolivar, on dead, partly corticated hardwood, coll. L. Ryvarden, 10.VI.1995 (O 37539); **Estado Amazonas**, Yutajé, on dead naked angiosperm wood, coll. L. Ryvarden, 12-19.VI.1997 (O 40434). **VIETNAM: Ha Noi**, Tam Dao National Park, 1050 m a.s.l., on bamboo trunk, coll. S.H. Wu & S.Z. Chen, 1.VII.1998, Wu 9807-15 (TNM F9007); on dead bamboo stem, coll. S.H. Wu & S.Z. Chen, 1.VII.1998, Wu 9807-16 (TNM F9008); **Ha Tay Province**, Ba Vi National Park, 650 m a.s.l., on bamboo stem, coll. S.H. Wu & S.Z. Chen, 4.VII.1998, Wu 9807-96 (TNM F9081).

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Caloplaca gyrophorica (lichenized *Ascomycota*), a new saxicolous lichen species from India

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Abstract — A gyrophoric acid-containing *Caloplaca* species is reported from India for the first time. *Caloplaca gyrophorica* was found growing over rocks in temperate regions of Neora Valley National Park, Darjeeling, India. It is compared with the other three known gyrophoric acid-containing *Caloplaca* species and with the morphologically similar *C. hueana.*
Key words — depside, parietin, taxonomy, *Teloschistaceae*

Introduction

Gyrophoric acid, an orcinol para-depside, is reported from a number of lichens, but its presence in *Caloplaca* Th. Fr. is so far confined to only three species: *C. bogilana* Y. Joshi & Hur, *C. subflavorubescens* Y. Joshi & Hur, and *C. brownlieae* S.Y. Kondr. et al. (Joshi et al. 2010; Lumbsch et al. 2011). While investigating a specimen from Neora Valley National Park in the Eastern Himalaya, the authors found gyrophoric acid along with parietin as major compound. This is the first time that gyrophoric acid has been reported from an Indian *Caloplaca* species. When compared with other known gyrophoric acid-containing *Caloplaca* species, the specimen was found to differ entirely from those and hence described as new to science.

Materials & methods

Studied specimens are deposited at BSA and LWG. Description and photographs of external morphology are based on air-dried material observed under a dissecting stereomicroscope (Nikon SMZ645). Sections were made with a razor blade under the stereomicroscope and mounted in lactophenol cotton blue. Anatomical descriptions

are based on these preparations under a compound microscope (Nikon Eclipse E200). Ascospores were measured at 400× in water mounts; only free ascospores lying outside the asci were measured. A K+ purple reaction indicates the absence of the parietin complex, and a K+ red reaction indicates its presence. Secondary metabolites were identified by TLC as described by White & James (1985) and Orange et al. (2001) using solvent systems C and D. Mycological terms follow Kirk et al. (2008).

Taxonomy

Caloplaca gyrophorica Jagadeesh, Y. Joshi & G.P. Sinha, **sp. nov.** FIG. 1

MycoBank MB 800479

Similis C. hueana *sed differt in non-inspersum hymenium, grandior sporae et C+ rubescens thallus (acidum gyrophoricum).*

TYPE: India. West Bengal: Darjeeling district, Kalimpong subdivison, Neora Valley National Park, Aloobari, 27°07′33.1″N 88°42′55.2″E, alt. 2479 m, on exposed siliceous rocks, 17 May 2008, T.A.M. Jagadeesh Ram 4356 (**holotype**, BSA; **isotype**, LWG).

ETYMOLOGY — The specific epithet refers to the presence of gyrophoric acid in the species.

THALLUS crustose, saxicolous, areolate to subsquamulose; areoles confluent to scattered, yellowish brown to grayish brown, yellowish gray (when wet), ± rounded to ellipsoid or irregular, 0.2–0.5 mm wide; cortex thin, 7.5 µm thick, paraplectenchymatous, necral layer absent; algal layer continuous; medulla hyaline, of densely interwoven hyphae, with crystals. HYPOTHALLUS brownish black, scattered throughout and between the areoles.

APOTHECIA numerous, scattered to ± aggregated, rounded to somewhat angular, sessile to adnate, distinctly constricted at base, 0.3–0.5(–0.8) mm diam., lecanorine; disc brownish orange, yellowish orange (when wet), plane to convex, epruinose; margin concolorous to thallus, thin, smooth, uneven by age, slightly emergent from the disc. EPIHYMENIUM yellowish brown, 10–15.5(–25) µm thick, K+ red. HYMENIUM hyaline, not inspersed, (49–) 52.5–60(–75) µm high, I+ blue. HYPOTHECIUM hyaline, (30–)75–87.5(–100) µm thick, paraplectenchymatous. THALLINE EXCIPULUM up to 90 µm thick, with algae, without crystals. PROPER EXCIPULUM hyaline, thin. PARAPHYSES branched and anastomosing, 1–1.5 µm wide, tips not swollen. ASCI clavate, 8-spored, 48–58 × 13–15 µm. ASCOSPORES biseriate, hyaline, ellipsoid, polarilocular, (10–)11.5–14(–16) × 7–10 µm, isthmus 2.5–3.75 µm. PYCNIDIA not seen.

CHEMISTRY — Spot test reactions: thallus K–, C+ red, KC+ red, P–. Epihymenium K+ red, C–. TLC: gyrophoric acid and parietin.

ECOLOGY & DISTRIBUTION — Found growing over exposed siliceous rocks at ca 2400 m in Neora Valley National Park, Eastern Himalaya. Temperatures range from subzero to 20°C. The hilly slopes are covered by bushy secondary vegetation with some parts cultivated prior to the 1992 park establishment.

FIGURE 1: *Caloplaca gyrophorica*. Habit. Scale bar = 5 mm.

REMARKS — *Caloplaca gyrophorica* is characterized by an areolate to subsquamulose grayish brown thallus, brownish orange lecanorine apothecia with concolorous margins, and gyrophoric acid and parietin as major chemical constituents.

Morphologically the new species is most likely to be confused with the saxicolous *Caloplaca hueana* B. de Lesd., which differs in its inspersed hymenium, much smaller spores (8.5–11(–12) × 5.1–8.5 µm; Wetmore 1996), tropical distribution, and lack of gyrophoric acid.

The saxicolous species *C. bogilana*, differs from *C. hueana* in a grayish areolate thallus, simple to furcate paraphyses with swollen tips, numerous pycnidia, a maritime distribution, and atranorin and lecanoric acid present in most collections (Joshi et al. 2010). *Caloplaca subflavorubescens* differs in a strictly corticolous habit, a squamulose to subfruticose yellowish green to grayish green thallus, septate paraphyses, and the presence of 7-chloroemodin, emodin, fragilin, and atranorin; moreover, gyrophoric acid is present only rarely and absent from the common *C. subflavorubescens* chemotype (Joshi et al. 2010). The saxicolous *C. brownlieae* differs from *C. hueana* in having pinkish orange or cinnabarine areoles and immersed to slightly erumpent apothecia and containing ovoic acid, lecanoric acid, xanthorin, and erythroglaucin (Lumbsch et al. 2011).

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Seven dark fruiting lichens of *Caloplaca* from China

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Abstract —Detailed taxonomic descriptions with photos are presented for seven dark fruiting *Caloplaca* species found in China. *Caloplaca albovariegata*, *C. atrosanguinea*, and *C. diphyodes* are apparently new to Asia, *C. bogilana*, and *C. variabilis* are new to China, and *C. conversa* and *C. pulicarioides* are new to northern China.

Key words —*Ascomycota*, *Teloschistales, Teloschistaceae*

Introduction

The large cosmopolitan genus *Caloplaca* (*Teloschistaceae*, *Teloschistales*, *Lecanoromycetes*, *Ascomycota*) was established by Theodor Fries in 1860 (Kirk et al. 2008). *Caloplaca* is characterized by a totally immersed to subfruticose crustose thallus, typically teloschistacean ascus, polarilocular ascospores, and various anthraquinones or no substances (Poelt & Pelleter 1984, Søchting & Olecha 1995). The genus reportedly comprises more than 500 species worldwide (Kirk et al. 2008), but only 59 species are known in China (Abbas et al. 1996, Aptroot & Seaward 1999, Arocena et al. 2003, Wei 1991, Xahidin et al. 2010, Zhao & Sun 2002).

During our study of *Lecanora* Ach. in China, six *Caloplaca* species with black apothecial discs and one with a red-brown apothecial disc were found. Of these, five species are new to China. Descriptions with photos of important characters of these species are provided here.

Materials & methods

The specimens studied are preserved in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University). Morphological and anatomical characters

were examined under a COIC XTL7045B2 stereomicroscope and Olympus CX41 polarizing microscope. Lichen substances were identified using the standardized thin layer chromatography techniques (TLC) with solvent system C (Orange et al. 2010). Photos were taken under Olympus SZX16 and BX61 with DP72.

Terms applied to *Caloplaca* apothecial structures of are shown below in Fig. 1.

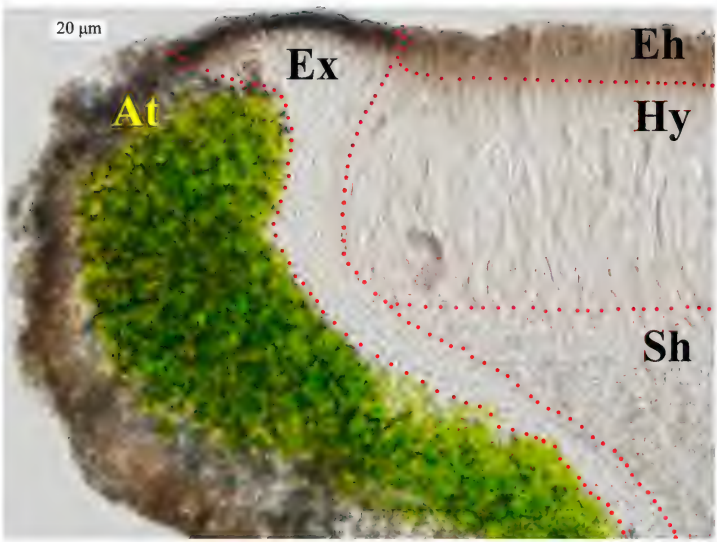

Figure 1. Terms applied to apothecial structures in *Caloplaca:* At = amphithecium; Ex = inner exciple; Eh = epihymenium; Hy = hymenium; Sh = subhymenium. Bar = 20µm.

Taxonomic descriptions

Caloplaca albovariegata (B. de Lesd.) Wetmore, Mycologia 86: 816 (1995 ["1994"]) Figs 2, 3

Morphology — Thallus saxicolous, crustose, greenish gray to blackish gray, stipitate areolate); cortex with crystals in some places; medulla with obvious crystals; algal layer clustered; prothallus not seen. Apothecia lecanorine, sessile; disc black, epruinose; inner exciple black at the top; amphithecium with very thick algal layer and obvious crystals; epihymenium brown, without crystals; hymenium and subhymenium hyaline. Ascus 8-spored; ascospores polarilocular, ellipsoid, 15–20 × 7.5–10 µm, isthmus 2–4.5 µm; paraphyses simple, with 1–2 somewhat swollen apical cells. Pycnidia not observed.

Chemistry — Epihymenium K+ light purple, C–; amphithecial cortex and top of inner exciple K+ purple, C+ purple. Thallus and medulla K–, C–, KC–, P–. Secondary metabolites: no lichen substances detected by TLC.

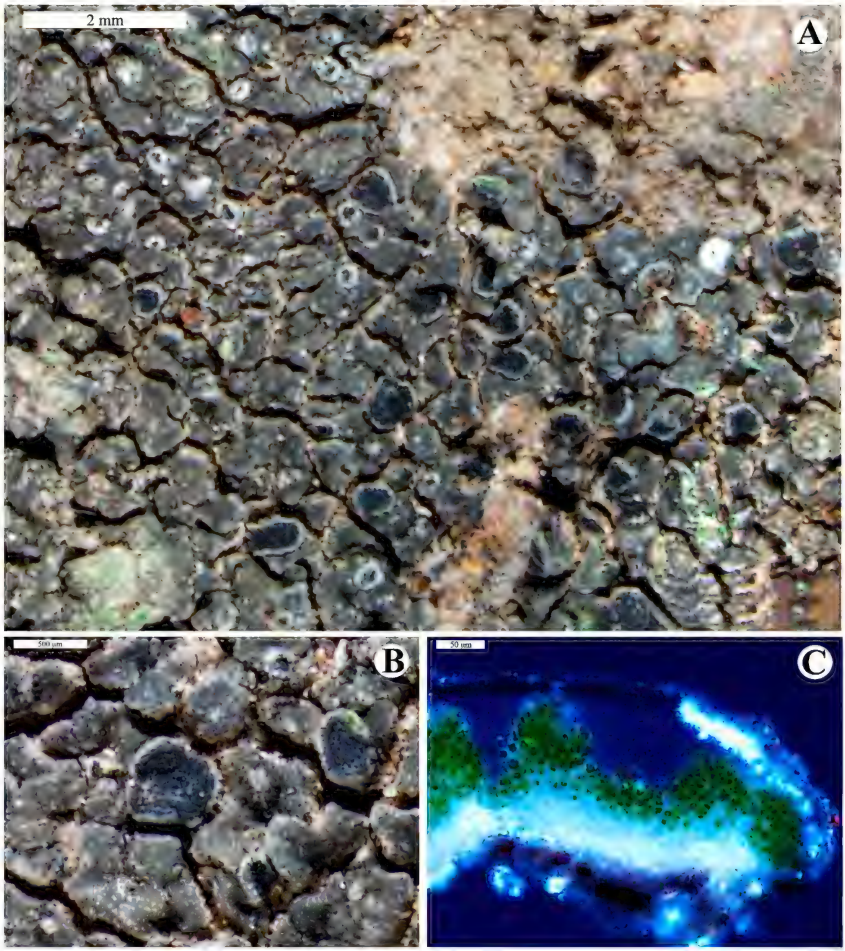

FIGURE 2. *Caloplaca albovariegata* (Wang 20124075, SDNU). A: Thallus; B: Apothecium; C: Thallus section showing clustered algal layer and crystals in cortex and medulla. Scale bars: A = 2 mm; B = 500 µm; C = 50 µm.

DISTRIBUTION — *Caloplaca albovariegata* has been reported from western North America (Wetmore 1995, 2007). New to China and Asia.

SPECIMEN EXAMINED: **CHINA. SHANXI,** NINGWU COUNTY, Mt. Luya, alt. 2500 m, on rock, 26 Aug. 2011, H.Y. Wang 20124075 (SDNU).

COMMENTS — *Caloplaca peliophylla* resembles *C. albovariegata* in the stipitate areolate thallus but differs in the shiny olive brown thallus and lighter brown apothecial discs. The similar *C. atroalba, C. diphyodes, C. ursina,* and *C. variabilis*

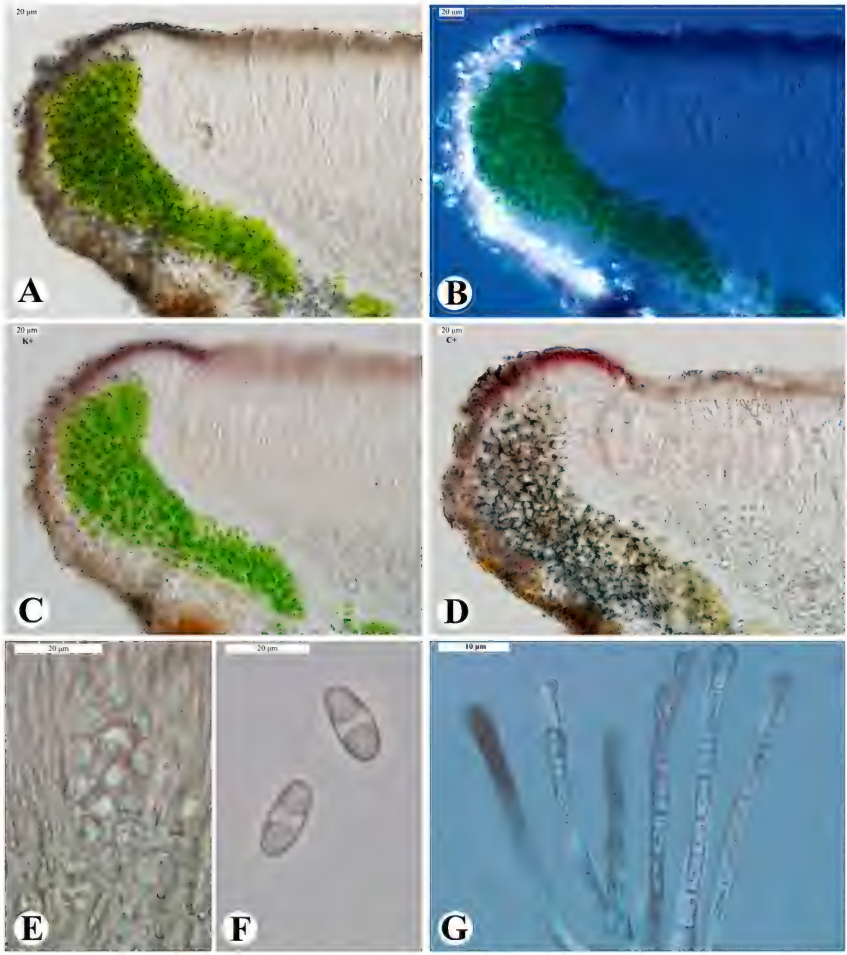

Figure 3. *Caloplaca albovariegata* (Wang 20124075, SDNU). A: Apothecium section; B: Amphithecium with obvious crystals; C: K+ reaction in epihymenium, amphithecial cortex, and top of inner exciple; D: C+ reaction in amphithecial cortex and top of inner exciple; E: 8-spored ascus; F: Polarilocular ascospores; G: Simple paraphyses with 1–2 somewhat swollen apices. Scale bars: A–F = 20 μm; G = 10 μm.

differ by having non-stipitate or only partly stipitate areolate thalli. See further discussion under *C. variabilis*.

Caloplaca atrosanguinea (G. Merr.) I.M. Lamb, Natl. Mus. Canada Bull. 132: 305 (1954) Figs 4, 5

Morphology — Thallus corticolous, crustose, light grey; cortex with indistinct crystals; medulla with obvious crystals; algal layer even; prothallus not

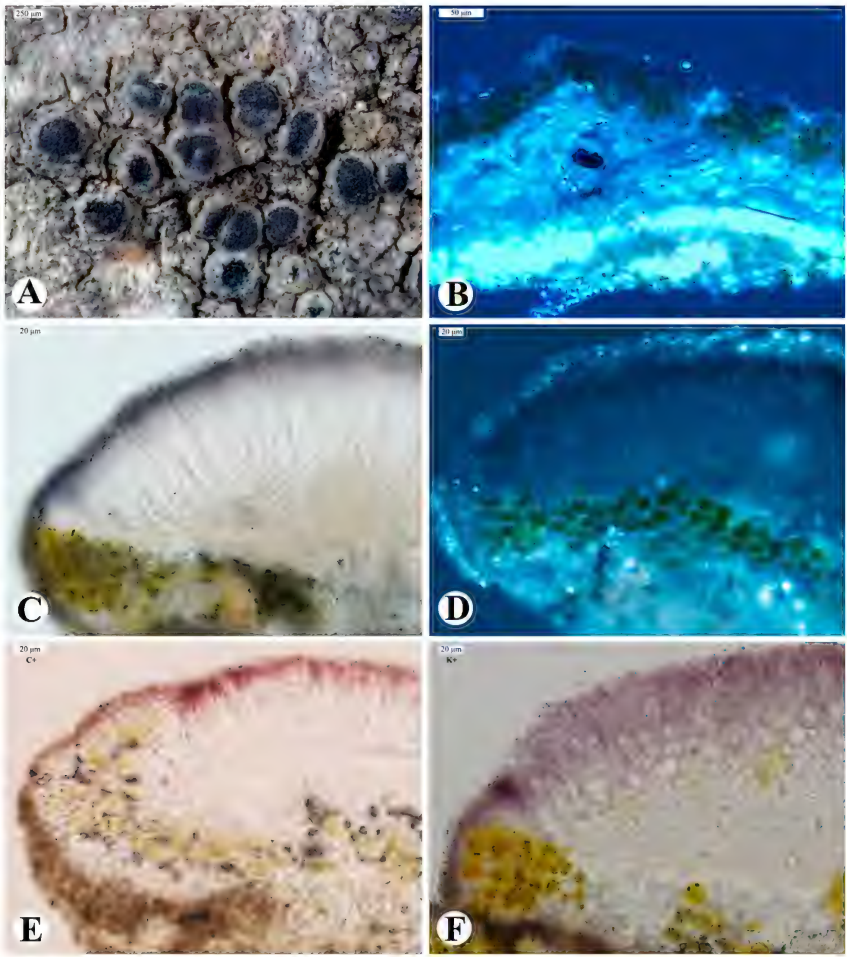

FIGURE 4. *Caloplaca atrosanguinea* (Zhao 20116843A, SDNU). A: Thallus; B: Even algal layer and crystals in cortex and medulla; C: Apothecium section; D: Crystals in amphithecium and epihymenium; E: C+ reaction in epihymenium and top of inner exciple; F: K+ reaction in epihymenium, amphithecial cortex, and top of inner exciple. Scale bars: A = 250 µm; B = 50 µm; C–F = 20 µm.

seen. Apothecia lecanorine, sessile; disc black or somewhat brown, epruinose; inner exciple black at the top; amphithecium with crystals; epihymenium dark black, with crystals; hymenium and subhymenium hyaline. Ascus 8-spored; ascospores polarilocular, ellipsoid, 10–12.5 × 6.2–7.5 µm, isthmus 2–3 µm; paraphyses simple to branched at the top, with 2–3 swollen apical cells. Pycnidia present, ostiole black; conidia bacilliform, 3.5–6.3 × 0.8–1 µm.

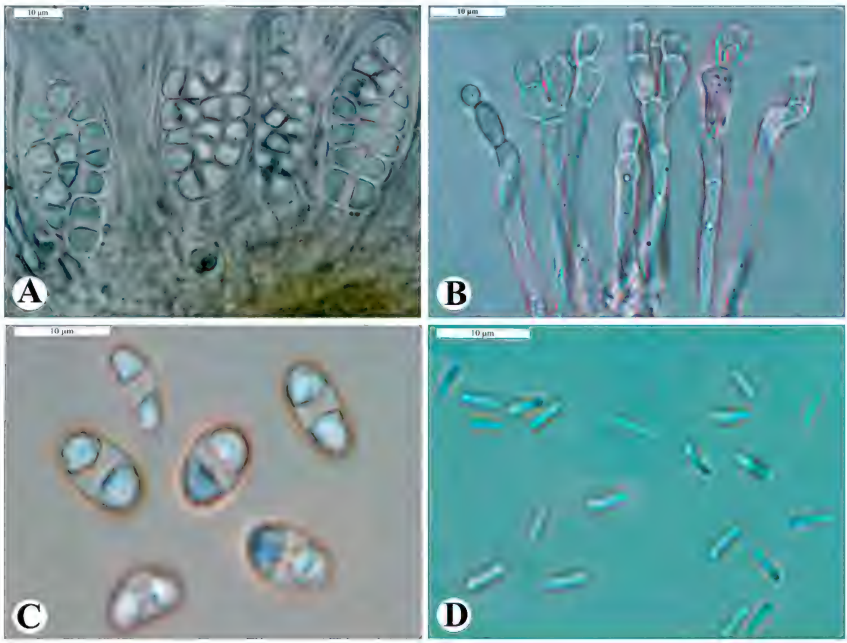

FIGURE 5. *Caloplaca atrosanguinea* (Zhao 20116843A, SDNU). A: 8-spored ascus; B: Simple to branched paraphyses with 2–3 swollen apical cells; C: Polarilocular ascospores; D: Bacilliform conidia. Scale bars = 10 μm.

CHEMISTRY — Epihymenium K+ reddish then purple, C+ reddish purple (FIG. 4E); top of inner exciple K+ reddish then purple, C+ reddish purple; amphithecial cortex K+ light purple, C–. Thallus and medulla K–, C–, KC–, P–. Secondary metabolites: no lichen substances detected by TLC.

DISTRIBUTION — *Caloplaca atrosanguinea* has been reported from North America (McCune 1984, Wetmore 1995, 2007). New to China and Asia.

SPECIMEN EXAMINED: **CHINA. XINJIANG**, URUMQI COUNTY, Nanshan-Bayi Forest farm, alt. 1775 m, on bark, 26 Jun. 2005, J.Z. Zhao 20116843A (SDNU).

COMMENTS — *Caloplaca parvula* resembles *C. atrosanguinea* in corticolous habitat and dark colored epihymenium but is distinguished by K– epihymenium and tissues and ascospores with a very narrow (1–1.5 μm) isthmus (McCune 1984). *Caloplaca floridana* and *C. pollinii* resemble *C. atrosanguinea* in corticolous habitat and the K+ purple epihymenium. However, *C. floridana* has an epihymenium without crystals and ascospores with a wider isthmus (5.5–7 μm; Wetmore 1995), and *C. pollinii* has a patchily yellow-brown hymenium and four lichen substances (parietin, emodin, fallacinol, fallacinal).

Caloplaca bogilana Y. Joshi & Hur, The Lichenologist 42: 716 (2010) FIGS 6, 7

MORPHOLOGY — Thallus saxicolous, crustose, areolate, grayish-white, glossy, thick; cortex and medulla with obvious crystals; algal layer even; prothallus not seen. Apothecia lecanorine, sessile; disc brown to black, epruinose; top of inner exciple concolorous with disc; amphithecium with obvious crystals; epihymenium orange-brown, with concolorous crystals; hymenium and subhymenium hyaline. Ascus 8-spored; ascospores polarilocular, ellipsoid, 10–

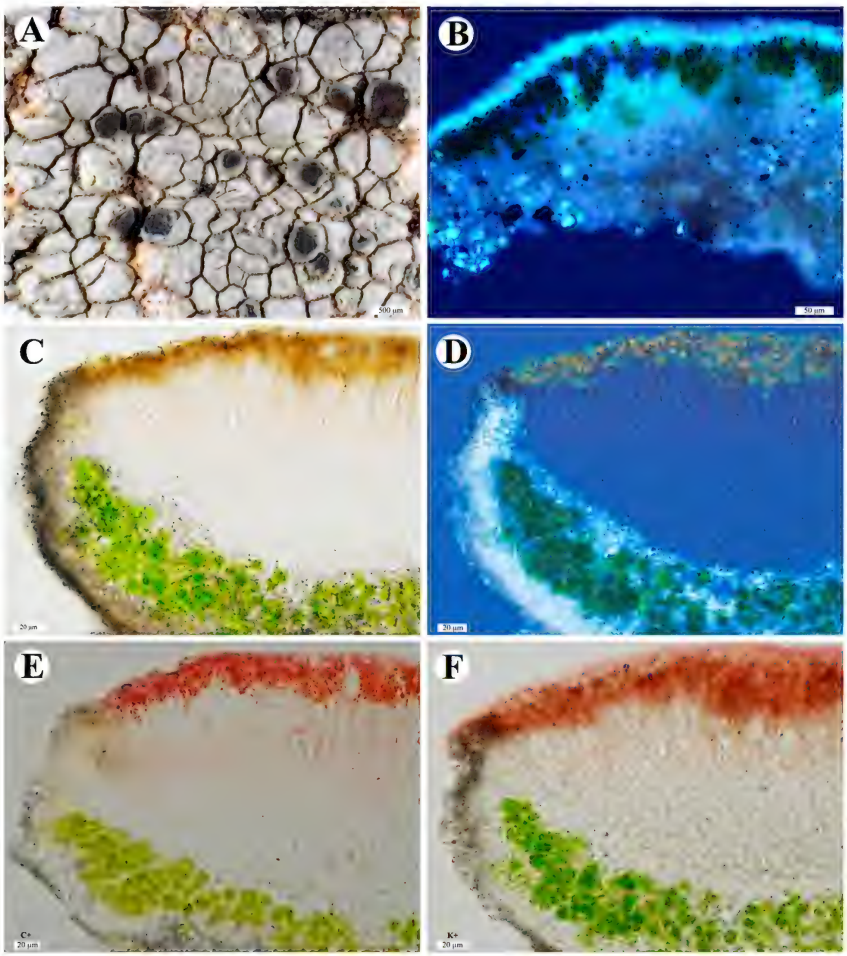

FIGURE 6. *Caloplaca bogilana* (Cheng 20120490B, SDNU). A: Thallus; B: Even algal layer and crystals in cortex and medulla; C: Apothecium section; D: Obvious crystals in amphithecium and orange-brown crystals in epihymenium; E: C+ reaction in epihymenium; F: K+ reaction in epihymenium. Scale bars: A = 500 μm; B = 50 μm; C–F =20 μm.

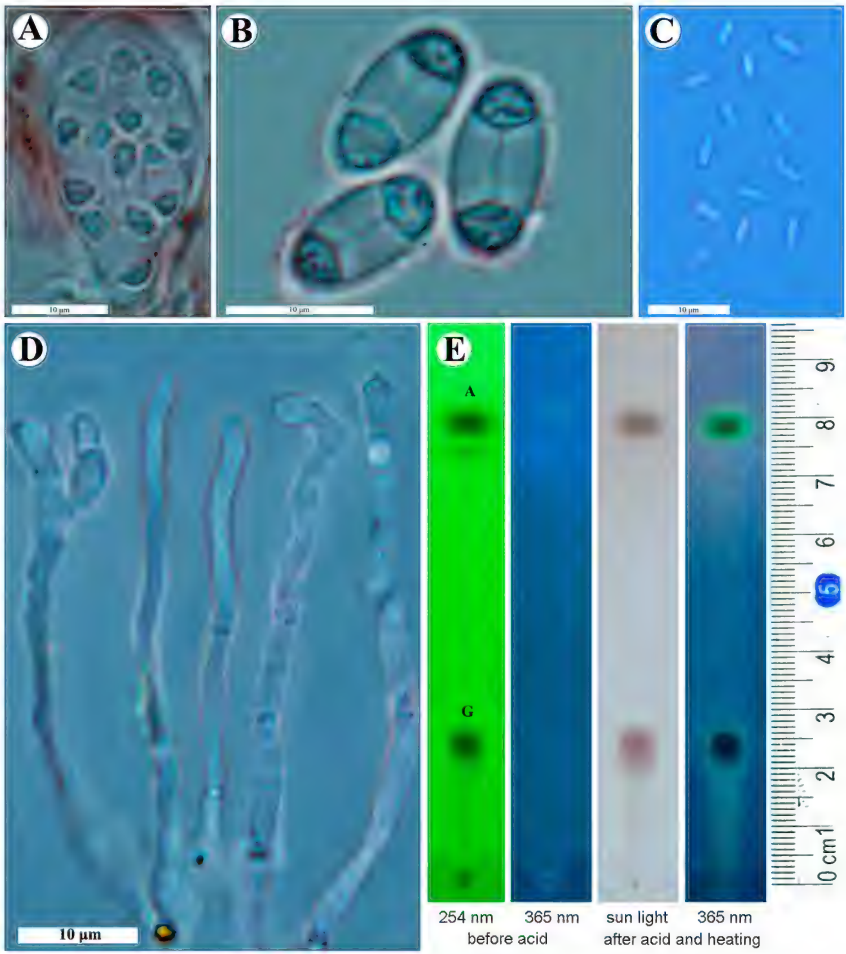

FIGURE 7 *Caloplaca bogilana* (Cheng 20120490B, SDNU). A: 8-spored ascus; B: Polarilocular ascospores; C: Long ellipsoid conidia; D: Simple to branched paraphyses; E: TLC result. Scale bars = 10 µm.

12.5 × 4.0–6.3 µm, isthmus 3.5–5.0 µm; paraphyses simple to branched at the top. Pycnidia numerous, ostiole black; conidia long ellipsoid, 3.0–4.5 × 1.1–1.6 µm.

CHEMISTRY — Epihymenium K+ red, C+ red; amphithecium and top of inner exciple K–, C–. Thallus and medulla K+ yellow, C+ red, KC+ red, P–. Secondary metabolites: atranorin, gyrophoric and lecanoric acids, ± parietin.

DISTRIBUTION — *Caloplaca bogilana* has been reported previously only from coastal areas of Bogil Island in South Korea (Joshi 2010), while we found it from both coastal and inland areas in China.

Specimens examined: **CHINA. Liaoning**, Beipiao city, Mt. Dahei, alt. 1300 m, on rock, 14 Aug. 2011, Y.L. Cheng 2012490B (SDNU), 20120489B (SDNU); **Shandong**, Yantai city, Mt. Kunyu, alt. 400 m, on rock, 12 Jun. 2010, Y.L. Cheng 20128005 (SDNU).

Comments — *Caloplaca subleptozona* resembles *C. bogilana* in having an areolate thallus and atranorin but differs in its biatorine apothecia with pruinose discs and UV+ thallus.

Caloplaca conversa (Kremp.) Jatta, Syll. Lich. Ital.: 254 (1900) Figs 8, 9

Morphology — Thallus saxicolous, crustose, ivory-white to grayish-white, areolate, pruinose; cortex and medulla with obvious crystals; algal layer even;

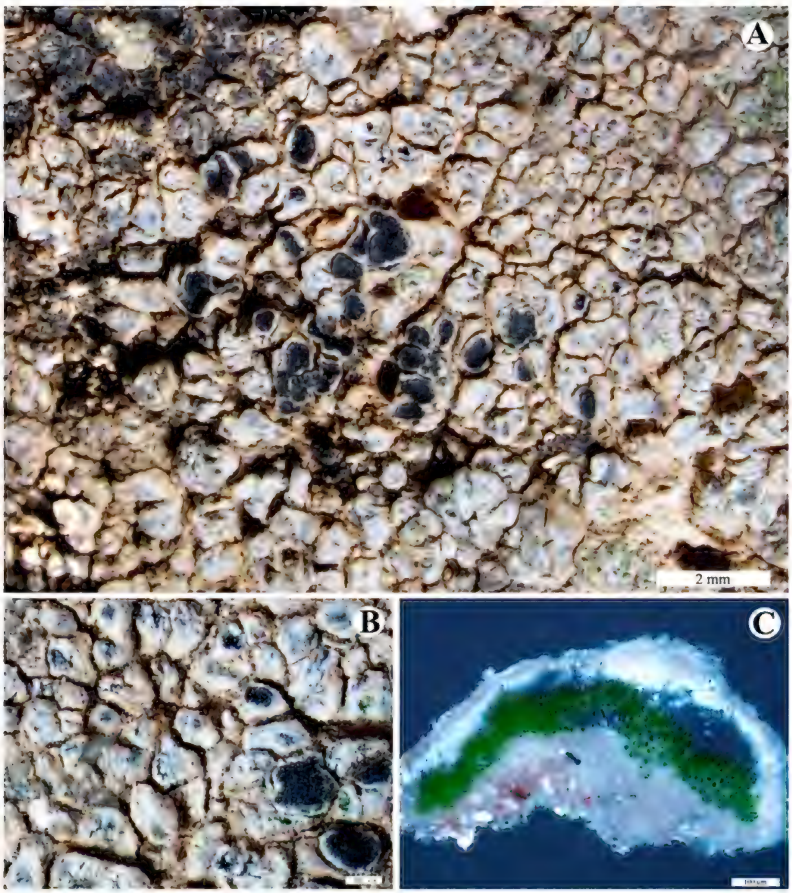

Figure8. *Caloplaca conversa* (Wang 20122965, SDNU). A: Thallus; B: Pruina and apothecia in thallus, showing; C: Even algal layer and crystals in cortex and medulla. Scale bars: A = 2 mm; B = 400 μm; C = 100μm.

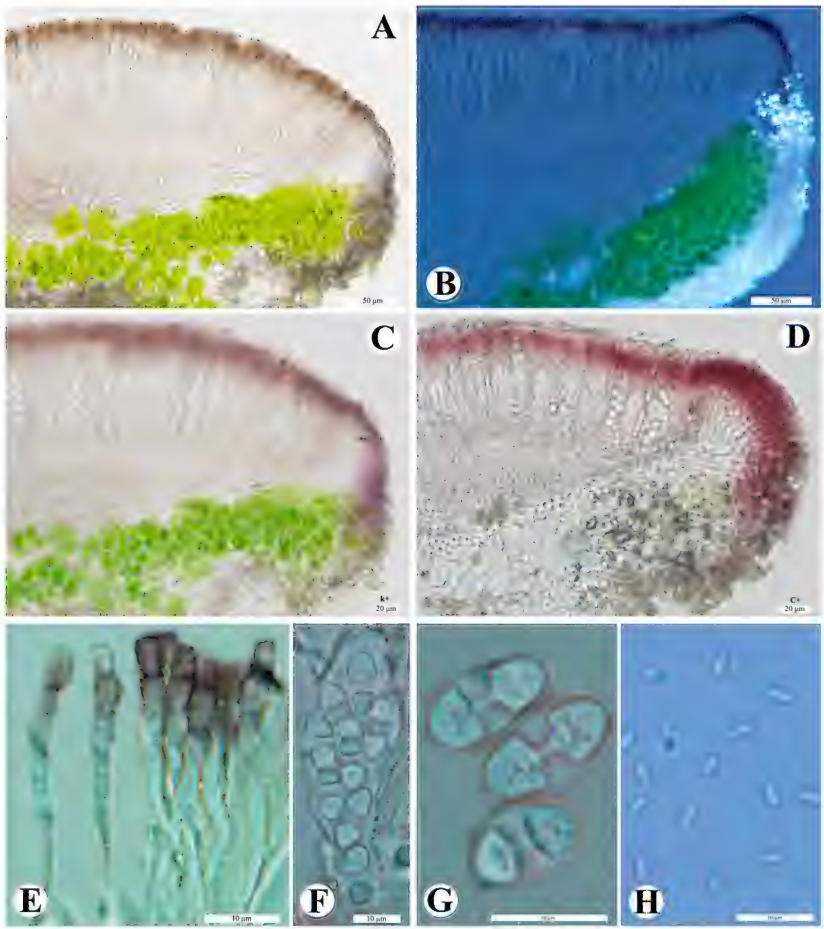

Figure 9. *Caloplaca conversa* (Wang 20122965, SDNU). A: Apothecium section; B: Crystals in amphithecium; C: K+ reaction in epihymenium, amphithecial cortex, and top of inner exciple; D: C+ reaction in epihymenium, amphithecial cortex, and top of inner exciple; E: Simple paraphyses with 4-–6 swollen apical cells; F: 8-spored ascus; G: Polarilocular ascospores; H: Pyriform conidia. Scale bars: A, B = 50 μm; C, D = 20 μm; E–H = 10 μm.

prothallus not seen. Apothecia lecanorine, immersed or adnate; disc black, epruinose; inner exciple black at the top; amphithecium with obvious crystals; epihymenium brown, without crystals; hymenium and subhymenium hyaline. Ascus 8-spored; ascospores polarilocular, ellipsoid, 10–15 × 4–6.5 μm, isthmus 2–4.5 μm (Figs 9F, G); paraphyses simple, with 4–6 swollen apical cells. Pycnidia present, ostiole black; conidia pyriform, 3–4 × 2–2.5 μm.

Chemistry — Epihymenium, top of inner exciple, and amphithecial cortex K+ weakly purple, C+ purple. Thallus and medulla K–, C–, KC–, P–. Secondary metabolites: no lichen substances detected by TLC.

Distribution — *Caloplaca conversa* has been reported from Europe, North America, and Hong Kong, China (Aptroot & Seaward 1999, Breuss 1988, Wetmore 1995, 2007). New to northern China.

Specimens examined: **CHINA. Inner Mongolia**, Ba Yan Hot, Mt. Helan, alt. 1500 m, on rock, 19 Aug. 2011, H.Y. Wang 20122965 (SDNU), 20122520 (SDNU); 19 Aug. 2011, P.M. Wang 20123717 (SDNU), 20123333 (SDNU); alt. 1800 m, on rock, 19 Aug. 2011, H.Y. Wang 20123141B (SDNU); alt. 2000 m, on rock, 19 Aug. 2011, D.B. Tong 20123046 (SDNU); Wulate Houqi, Mt. Helan, alt. 1600 m, on rock, 16 Aug. 2011, D.B. Tong 20123522 (SDNU), 20122837 (SDNU), 20122460 (SDNU), 20123568 (SDNU), 20123471 (SDNU); 17 Aug. 2011, P.M. Wang 20123635 (SDNU), 20123618 (SDNU), 20123902B (SDNU), 20123109 (SDNU); 17 Aug. 2011, X.R. Kou 20123673 (SDNU); alt. 1700 m, on rock, 17 Aug. 2011, X. R. Kou 20123898 (SDNU), 20123676 (SDNU); Wulate Houqi, Mt. Hebashige, alt. 1600 m, on rock, 16 Aug. 2011, H.Y. Wang 20123003 (SDNU), 20123017 (SDNU), 20122966 (SDNU), 20123977 (SDNU), 20123518A (SDNU).

Comments — *Caloplaca alociza* and *C. chalybaea* resemble *C. conversa* in black sunken apothecia. However, *C. alociza* has ± immersed thallus and larger ascospores (15–18 × 7–8 µm) (Wilk & Flakus 2006), while *C. chalybaea* has a black-brown to lead-grey thallus with conspicuous black prothallus and mature apothecia with blue pruina. The also similar *C. conciliascens* differs in having an indistinct thallus, lecideine apothecia with brownish red discs, and a blackish green top of inner exciple.

Caloplaca diphyodes (Nyl.) Jatta, Syll. Lich. Ital.: 259 (1900) Figs 10, 11

Morphology — Thallus saxicolous, crustose, off-white, areolate; cortex with somewhat crystals; medulla with obvious crystals; algal layer even; prothallus not seen. Apothecia lecanorine, sessile; disc black, epruinose; inner exciple black at the top; amphithecium with obvious crystals; epihymenium dark brown, without crystals; hymenium and subhymenium hyaline. Ascus 8-spored; ascospores polarilocular, ellipsoid, 12.5–15 × 6–8 µm, isthmus 3–4 µm; paraphyses simple, with 3–6 swollen apical cells. Pycnidia present, usually on marginal areoles, ostiole black, margin white; conidia filiform, 15–17.5× 0.6–0.7 µm.

Chemistry — Epihymenium, top of inner exciple, and amphithecial cortex K+ purple, C+ weakly purple. Thallus and medulla K–, C–, KC–, P–. Secondary metabolites: no lichen substances detected by TLC.

Distribution — *Caloplaca diphyodes* has been reported from Europe (Coste 2005, Magnusson 1937). New to China and Asia.

Specimens examined: **CHINA. Inner Mongolia**, Ba Yan Hot, Mt. Helan, alt. 1300m, on rock, 19 Aug. 2011, H.Y. Wang 20122508 (SDNU), 20122519 (SDNU), 20122615

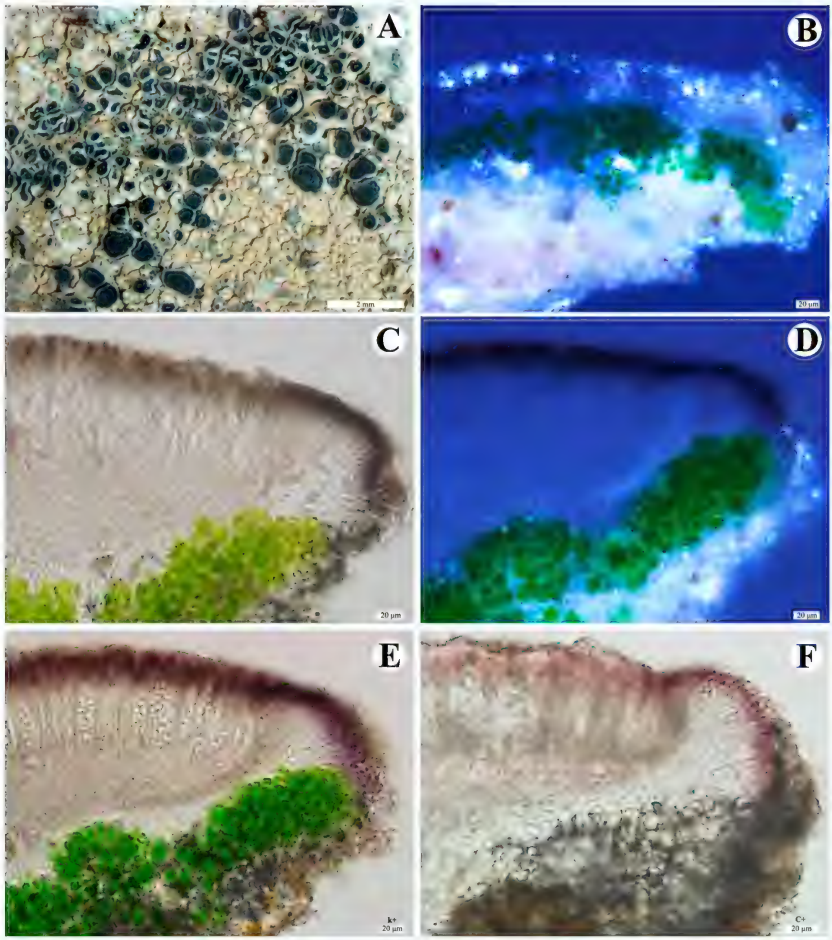

Figure 10. *Caloplaca diphyodes* (Wang 20122508, SDNU). A: Thallus; B: Even algal layer and crystals in cortex and medulla; C: Apothecium section; D: Crystals in amphithecium; E: K+ reaction in epihymenium, amphithecial cortex, and top of inner exciple; F: C+ reaction in epihymenium, amphithecial cortex, and top of inner exciple. Scale bars: A = 2 mm; B–F = 20 μm.

(SDNU), 20122664A (SDNU), 20123015A (SDNU), 20123253 (SDNU); 19 Aug. 2011, P.M. Wang 20123824A (SDNU); 19 Aug. 2011, X.R. Kou 20123879 (SDNU); 19 Aug. 2011, D.B. Tong 20122781A (SDNU).

Comments — *Caloplaca atroalba* and *C. ursina* resemble C. *diphyodes* in a nonstipitate areolate thallus and entirely raised black epruinose apothecia. However, *C. atroalba* has ascospores with a narrower isthmus (1.5–3 μm; Wetmore 1995), paraphyses with slightly swollen apical cells, and a weakly K+

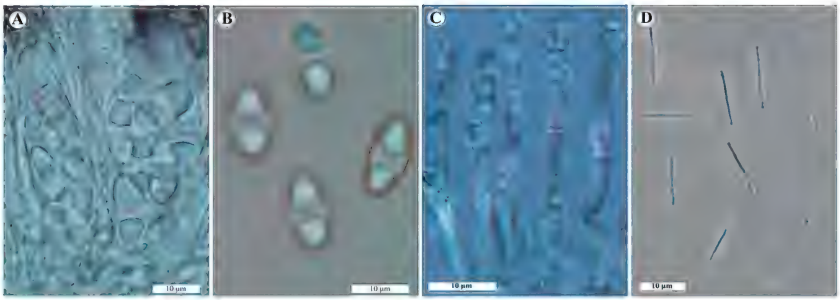

FIGURE 11. *Caloplaca diphyodes* (Wang 20122508, SDNU). A: 8-spored ascus; B: Polarilocular ascospores; C: Simple paraphyses with 3–6 swollen apical cells; D: Filiform conidia. Scale bars = 10 μm.

purple epihymenium while *C. ursina* has a K– epihymenium and amphithecial cortex.

Caloplaca pulicarioides Aptroot, Trop. Bryol. 17: 69 (1999) FIG. 12

MORPHOLOGY — Thallus corticolous, crustose, white to greenish white, areolate; cortex with indistinct crystal; medulla with obvious crystals; algal layer even; prothallus not seen. Apothecia lecanorine, adnate to immersed; disc brown to red-brown, epruinose; top of inner exciple concolorous with disc; amphithecium with obvious crystals; epihymenium brown, without crystals; hymenium and subhymenium hyaline. Ascus 8-spored; ascospores polarilocular, ellipsoid, 7.5–10 × 4–5 μm, isthmus 1.5–2.5 μm; paraphyses simple to branched at the top, without swollen cells. Pycnidia present, numerous, ostiole brown to red-brown; conidia bacilliform, 2.5–3.5 × 0.8–1.2 μm.

CHEMISTRY — Epihymenium, top of inner exciple, and amphithecium K–, C–. Thallus and medulla K–, C–, KC–, P–. Secondary metabolites: two unknown substances (S-1, S-2) detected by TLC.

DISTRIBUTION — *Caloplaca pulicarioides* has been reported from Africa and Hong Kong, China (Aptroot & Seaward 1999, Seaward & Aptroot 2003). New to northern China.

SPECIMENS EXAMINED: **CHINA. SHANDONG**, RONGCHENG COUNTY, Mt. Chengshan, alt. 50 m, on bark, 20 Jul. 2011, L. Li 20116198 (SDNU), 20127916 (SDNU), 20116529 (SDNU), 20116525A (SDNU), 20116544A (SDNU), 20116630A (SDNU); 20 Jul. 2011, H.Y. Wang 20116103A (SDNU), 20116537A (SDNU), 20116538B (SDNU), 20116534A (SDNU), 20116533 (SDNU), 20116532 (SDNU), 20116199 (SDNU), 20116535 (SDNU); YANTAI CITY, Mt. Kunyu, alt. 300 m, on bark, 9 Jun. 2010, Y.L. Cheng 20127923 (SDNU).

COMMENTS — *Caloplaca pulicarioides* superficially resembles species of the *Lecanora pulicaris*-group, but its polarilocular ascospores place it in *Caloplaca*.

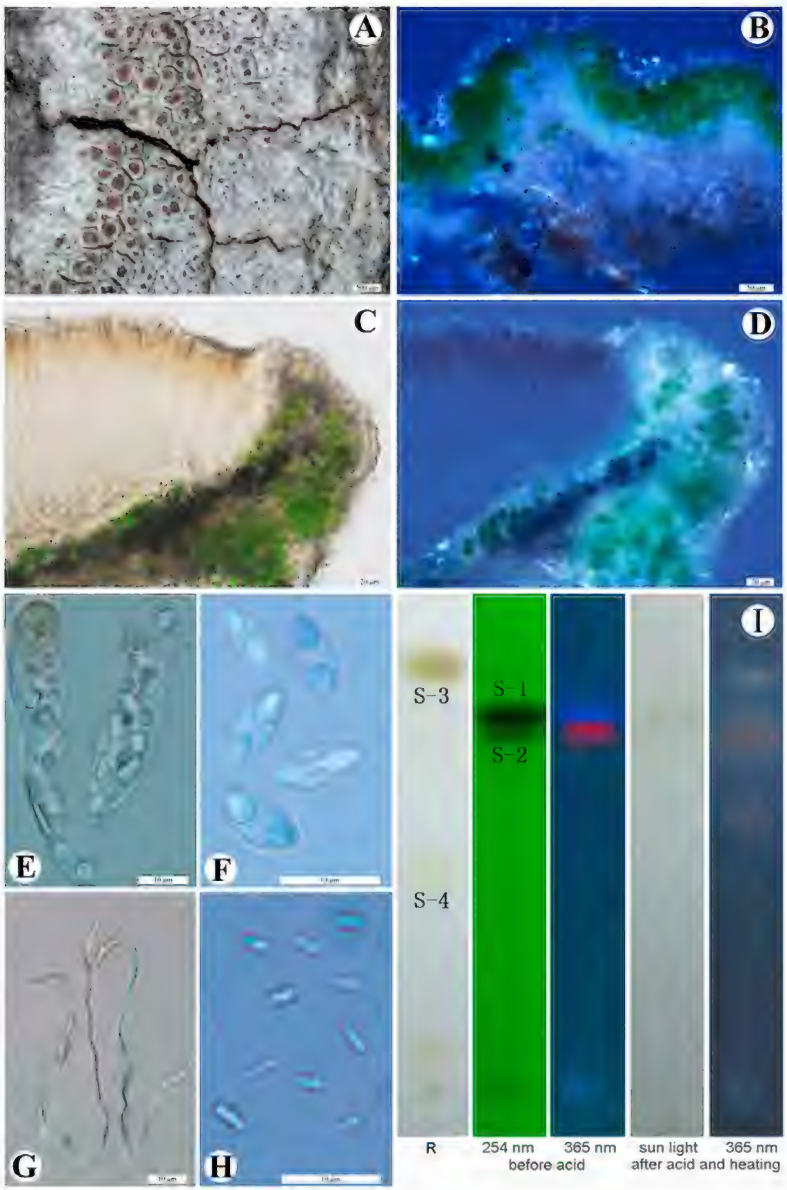

FIGURE 12. *Caloplaca pulicarioides* (Li 20116198, SDNU). A: Thallus; B: Even algal layer and crystals in cortex and medulla; C: Apothecium section; D: Crystals in amphithecium; E: 8-spored ascus; F: Polarilocular ascospores; G: Simple to branched paraphyses without swollen cells; H: Bacilliform conidia; I: TLC result (S-3 = Atranorin; S-4 = Norstictic acid). Scale bars: A = 500 μm; B = 50 μm; C, D = 20 μm; E–H = 10 μm.

The morphologically similar *C. homologa* and *C. trilocularis* differ in their consistently 3-loculate ascospores.

Caloplaca variabilis (Pers.) Müll. Arg., Mém. Soc. Phys. Hist. nat. Genève 16(2): 387 (1862) Figs 13, 14

Morphology — Thallus saxicolous, crustose, gray to blackish gray, partly stipitate areolate; cortex with crystals in some places; medulla with crystals; algal layer clustered; prothallus not seen. Apothecia lecanorine, sessile, round

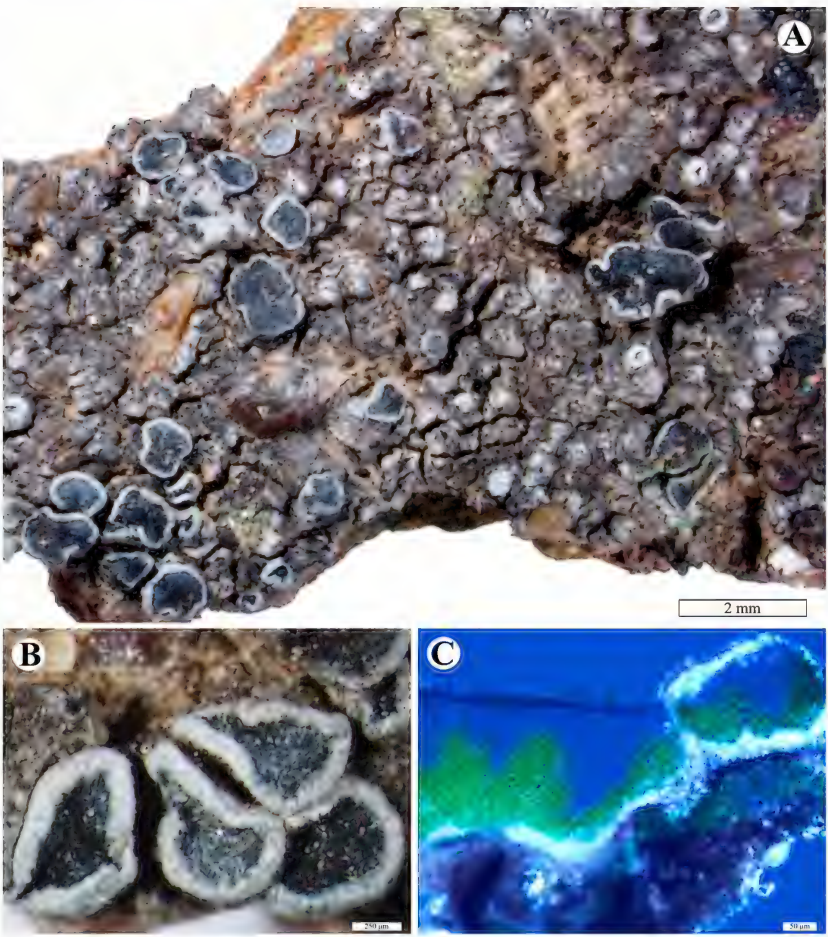

Figure 13. *Caloplaca variabilis* (Wang 20124114, SDNU). A: Thallus; B: Apothecial margin with thick pruina; C: Clustered algal layer and crystals in cortex and medulla. Scale bars: A = 2 mm; B = 250 μm; C = 50 μm.

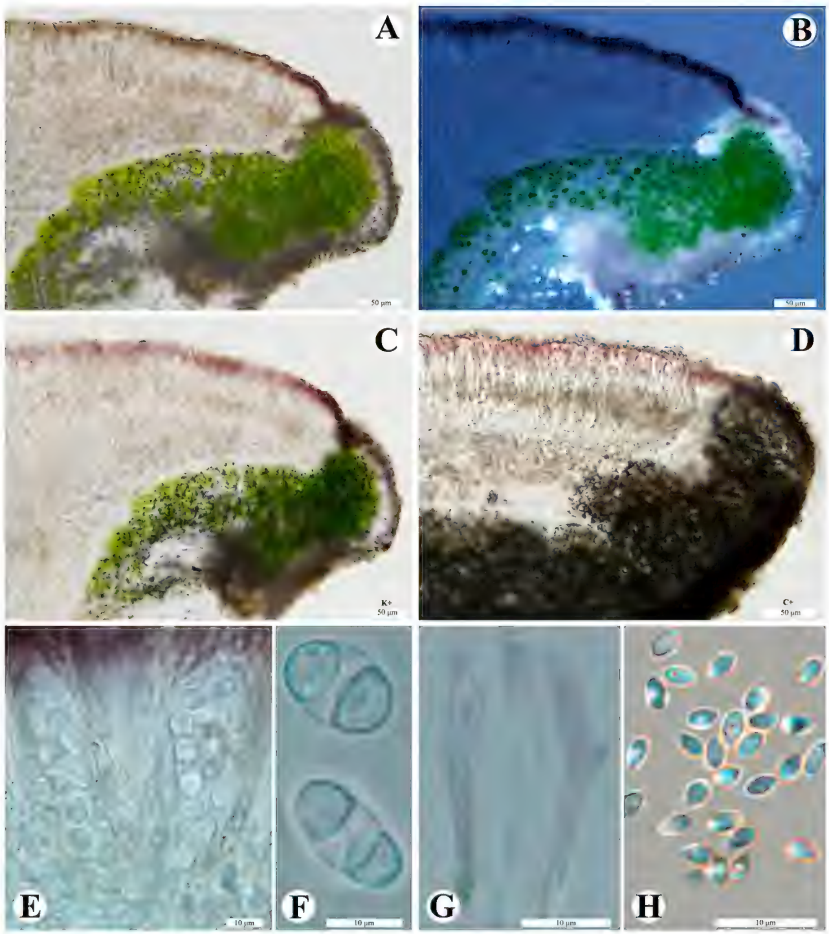

FIGURE 14. *Caloplaca variabilis* (Wang 20124114, SDNU). A: Apothecium section; B: Crystals in amphithecium; C: K+ reaction of epihymenium and top of inner exciple; D: C+ reaction of epihymenium and top of inner exciple; E: 8-spored ascus; F: Polarilocular ascospores; G: Simple to branched paraphyses with 2–4 somewhat swollen apical cells; H: Pyriform conidia. Scale bars: A–D = 50 µm; E–H = 10 µm.

to angular; disc black, sometimes pruinose; top of inner exciple concolorous with disc; amphithecium with thick pruina, thick algal layer, and crystals; epihymenium dark brown, without crystals; hymenium and subhymenium hyaline. Ascus 8-spored; ascospores polarilocular, ellipsoid, 15–20 × 7.5–10 µm, isthmus 2–3 µm; paraphyses simple to branched at the top, with 2–4 somewhat swollen apical cells. Pycnidia present, numerous, ostiole black; conidia pyriform, 3–4× 1.5–2 µm.

Chemistry — Epihymenium and top of inner exciple K+ weakly purple, C+ weakly purple; amphithecium K–, C–. Thallus and medulla K–, C–, KC–, P–. Secondary metabolites: no lichen substances detected by TLC.

Distribution — *Caloplaca variabilis* has been reported from Asia, Europe, and North America (Fryday 2004, Wetmore 1995, 2007, Yazıcı et al. 2008). New to China.

Specimen examined: **CHINA. Shanxi,** Ningwu county, Mt. Luya, alt. 2500 m, on rock, 26 Aug. 2011, H.Y. Wang 20124114 (SDNU).

Comments — *Caloplaca atroalba*, *C. diphyodes,* and *C. ursina* resemble *C. variabilis* but differ in their nonstipitate areolate thalli and non-pruinose apothecia. *Caloplaca atroalba* and *C. diphyodes* have even algal layers, and *C. atroalba* is further distinguished by ascospores with a poorly formed isthmus. *Caloplaca ursina* has a K– epihymenium and thallus cortex.

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Boletus atlanticus sp. nov., a new species of section *Luridi* from coastal dunes of NW Spain

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Abstract—*Boletus atlanticus,* found in coastal sand dunes in Galicia (NW of Spain) under *Cistus salviifolius* and maritime pines (*Pinus pinaster*), is described as a new species from section *Luridi* based on morphology. Morphological description, drawings of microscopic characters, and color pictures of the basidiomata are presented. A discussion about other close taxa of section *Luridi* is also outlined.

Key words— *Basidiomycota, Boletales,* taxonomy, Iberian Peninsula, Europe

Introduction

Mycological studies carried out in recent decades in the dunes ecosystems of Galicia (NW of Spain, Iberian Peninsula) along the Atlantic coast in sand-dominated areas with natural coastal vegetation and forested mainly with maritime pine (*Pinus pinaster* Aiton) have revealed several new species to science, including *Gyroporus ammophilus* (Castro & Freire 1989, 1995), *Agaricus freirei* (Blanco-Dios 2001), and *Leucocoprinus castroi* (Blanco-Dios 2003). Recently, in tertiary or gray dunes with vegetation dominated by *Cistus salviifolius* L. (*Cistaceae*) and a few maritime pines, several specimens of a *Boletus* species were collected. We determined from a detailed morphological study that the specimens belong in sect. *Luridi* Fr. based on their orange pores and variable blueing of the exposed yellow flesh.

After extensively reviewing the European and extra-European species of section *Luridi*, we concluded that this taxon does not correspond to any previously described species and describe it as a new species: *Boletus atlanticus.*

Materials & methods

The specimens were collected, documented and preserved using standard methods. Morphological descriptions are based on fresh material and analysis of photographs of fresh specimens. Microscopic observations were recorded both from fresh material and dried specimens rehydrated in 3% KOH. We used a solution of 1% Congo Red in water to observe cystidia (pleuro-, cheilo-, and caulocystidia) and Melzer's reagent to examine basidiospores, pileipellis and stipitipellis. Spore size is presented as (Min)(mean – SD)–(mean + SD)(Max), where Min = the lowest value measured, Max = the highest value, followed by the mean spore lengths and widths (Xm); Q = spore length : width ratios; and the mean volume (Vm) was determined using the formula $Vm = 4\pi/3\ a^2b$, where **a** is the radius of the minor axis and **b** the radius of the major axis (Breitenbach & Kränzlin 1991).

The maximum and minimum values and the length/width ratio (Q) of the pileipellis terminal cells were determined by sampling between the centre and the margin (Ladurner & Simonini 2003) in the superior layer of young sporocarps, as recommended by Singer (1965) and Muñoz (2005). Microscopical structures were documented by color microphotographs and line drawings on a light microscope equipped with a digital camera and a drawing tube device. The collected material has been deposited in the mycological herbarium LOU-Fungi (Centro de Investigación Forestal de Lourizán, Pontevedra, Spain).

Taxonomy

Boletus atlanticus Blanco-Dios & G. Marques, **sp. nov.** PLATE 1, FIG. 1

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Boletus luridus *similis sed differt pileo ab rosaceo ad aurantiaco vel flavo et margo cremeo, pori primum aurantiaco et ochraceo, denique aurantiaco-ferrugineo, stipes ut cuticula flava, nisi in inferiore tertio, purpureo-rubro, sporis leviter plus grandis, caulocystidiis multiformis.*

TYPE: Spain, A Coruña, Santa Uxía de Ribeira, Aguiño, near of Penisqueira beach, 29TMH9708, 10 m, in tertiary or gray dunes, under *Cistus salviifolius* and *Pinus pinaster*, 29.X.2005, leg. F. Romano, J. Sampedro, A. Sampedro & J.B. Blanco-Dios (**Holotype**, LOU-Fungi 19418).

ETYMOLOGY: the species epithet refers to the Atlantic Ocean coast of NW Spain where the specimens were found.

PILEUS 65–155 mm broad, hemispheric to convex in age, smooth, glabrous, slightly viscid in wet weather; more intensely coloured when young, ranging from pink to orange on the disc, fading with age, becoming light brown on the disc and with various shades of pink, orange and yellow toward the cream colored margin, margin undulate and slightly projecting. When bruised, pileus surface changes to deep blue, greyish blue or greenish and, finally, to black blue. HYMENOPHORE tubulose, tubes 8–15 mm long, adnate, yellow, changing to blue slowly when bruised or exposed. Pores small, compressed, oval (more abundant, 0.4–0.7 mm per pore) or triangular (less numerous and larger,

Plate. 1. *Boletus atlanticus* (holotype). Habit.
Photo by Jaime B. Blanco-Dios.

1.2–1.4 mm per pore), pale orange to yellow ochre when young, becoming orange to rust at maturity, staining intense greenish gray when handled. Stipe 60–130 × 15–55 mm, usually shorter than the diameter of the pileus, attenuate, napiform or fusiform, bright yellow except in the basal third that is reddish to purple, with a long and irregular orange reticulum, except in the upper portion of the stipe (1–2 cm long) where the reticulum is yellow and very fine. Stipe surface changes to blue or grayish blue when bruised. Context firm, soft at maturity, yellow, changing to blue, grayish blue or greenish blue when exposed, except in the lower part where garnet and purple colours dominate. Smell agreeable, more intense in the pileus, generally not distinctive or, sometimes, with garlic smell. Taste agreeable with unctuous texture. Edibility unknown. Chemical reactions: Melzer's reagent: positive in the flesh (deep blue); formol (35%): grayish-blue in the pileus, pores and stipe, almost not visible in the flesh; FeSO4 (10%): gray to greenish in the pileus, stipe and flesh, predominantly gray in the pores. Spore print deep olive-brown.

Basidiospores (10.5–)11.5–14(–15.5) × (4.5–)5.2–6.7(–7.3) μm, Xm = 12.7 × 6 μm, Q = 1.6–2.6(–3), Vm = 240 μm^3, (n = 100), ellipsoid to fusoid, smooth, guttulate, thick-walled, ochre gray in 3% KOH, dextrinoid in Melzer's. Basidia 20.5–35 × 5.5–14.5 μm, 4-spored, rarely 2-spored, sterigmata 2.5–3.5

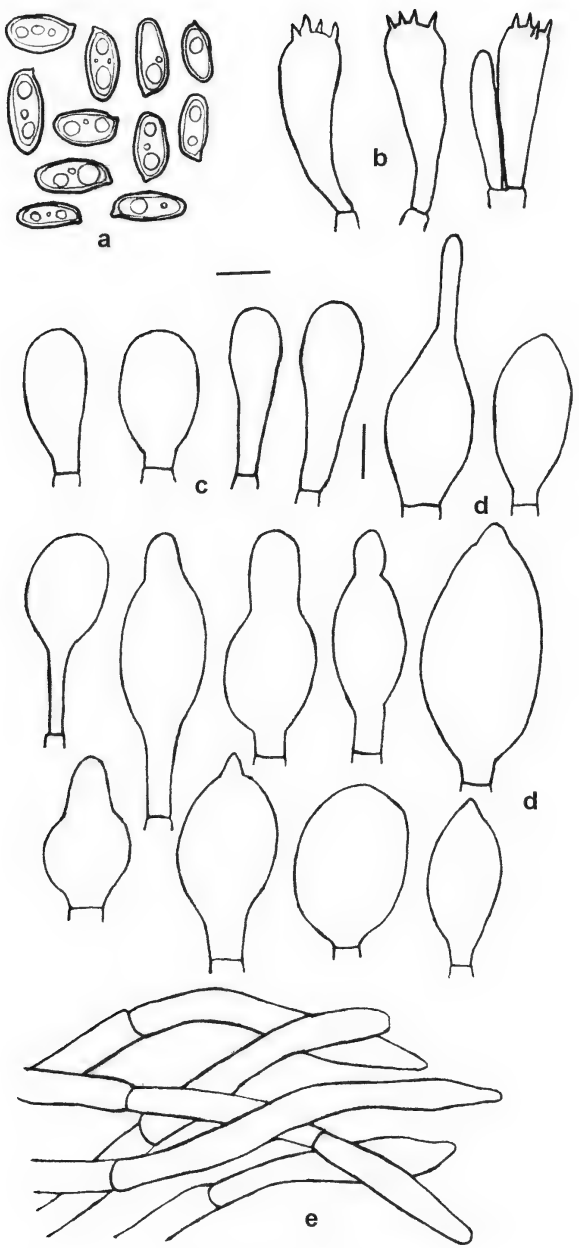

Fig. 1. *Boletus atlanticus* (holotype).
a. basidiospores; b. basidia; c. cheilocystidia; d. caulocystidia; e. pileipellis. Scale bars = 10 µm.

µm long, clavate, numerous basidioles. CHEILOCYSTIDIA AND PLEUROCYSTIDIA 18–37 × 6.5–13 µm, smooth and thin-walled, clavate or sphaeropedunculate, ochre, brown, orange to gray in 3% KOH. PILEIPELLIS a trichoderm consisting of interwoven hyphae 2.5–7 µm broad, long, subcylindric, septate, thin-walled, with brown or gray-brown intracellular pigment in 3% KOH and ochre or ochre-gray in Melzer's; terminal cells 33.5–73 × 4–7.5 µm, Q = 5.3–12.7, long, subcylindric to subfusiform with a rounded or acuminate apex. STIPITIPELLIS consisting of interwoven hyphae 1.5–6.5 µm wide, ochre, brown to gray in 3% KOH and in Melzer's. CAULOBASIDIA 24.5–55.5 × 6.5–17 µm, 4-spored, rarely 2-spored, sterigmata 3–6 µm long, clavate. CAULOCYSTIDIA 28.5–52.5 × 10–21 µm, smooth and thin-walled, versiform: clavate, fusiform-capitate, lageniform, sphaeropedunculate, pyriform-mucronate or urceolate, ochre, brown, orange to gray in 3% KOH. BASAL HYPHAE of stipe with a strong positive reaction amyloid. No CLAMP CONNECTIONS observed.

ECOLOGY AND DISTRIBUTION- On coastal sand dunes under *Cistus salviifolius* and maritime pines (*Pinus pinaster)*. Known only from a single locality in Spain. October-November.

ADDITIONAL COLLECTIONS EXAMINED: **SPAIN: A CORUÑA:** SANTA UXÍA DE RIBEIRA, Aguiño, near of Penisqueira beach, 29TMH9708, 10 m, in tertiary or gray dunes, under *Cistus salviifolius* and *Pinus pinaster*, 28.X.2005, leg. F. Romano, J. Sampedro & A. Sampedro (LOU-Fungi 19417); 5.XI.2005, leg. J.B. Blanco-Dios, E.M. Boullosa, I.M. Blanco-Boullosa & M.E Blanco-Boullosa (LOU-Fungi 19419); 11.XI.2008, leg. J.B. Blanco-Dios (LOU-Fungi 19434).

COMMENTS. Possession of orange pores and flesh changing to blue with variable intensity when sectioned places *Boletus atlanticus* in section *Luridi* (Muñoz 2005). This new species is morphologically characterized by a combination of (1) pileus color variably pink, orange, salmon, and yellow with a cream margin, (2) ovoid or triangular pores, (3) bright yellow stipe with the basal third reddish to purple and covered by an orange reticulum from apex to base except at the top of the upper yellow portion, (4) stipe context varying from garnet to purple in the basal third to half or basal third, (5) the pileus, stipe, and flesh staining gray to green (gray in the pores) with $FeSO_4$, (6) habitat in coastal dunes under *Cistus salviifolius* and *Pinus pinaster,* (7) spore Q = 1.6–2.6(–3) and Vm = 240 μm^3, and (8) versiform caulocystidia.

Among the morphologically similar species in sect. *Luridi* with a similarly colored pileus, orange or red pores, stipe with or without reticulum, and context bluing quickly and intensely when bruised or exposed, the closest European taxa are *B. comptus* Simonini, *B. luridus* Schaeff. s.l., *B. luteocupreus* Bertéa & Estadès, and *B. rhodopurpureus* Smotl. f. *rhodopurpureus.*

Boletus comptus differs from *B. atlanticus* in pileus color (variably gray, brown, pink, red, orange, and yellow), reticulum absent or limited to the stipe apex (and then variably yellow, orange, red, and brown), the basal stipe punctate

with reddish brown spots, red mature pores, exposed flesh reddening (and then only on the stipe) or not changing color, and habitat in *Quercus* spp. forests. Microscopically, the basidiospores of *B. comptus* are smaller ((9.5–)10.5–13.1 (–14.8) × (4.3–)4.8–5.9(–6.5) µm) and the caulocystidia are predominantly pyriform or clavate (Simonini 1992, Muñoz 2005, Péric & Péric 2006).

Boletus atlanticus should also be compared with *B. luridus* s.l., widespread in Europe and also reported for Africa, Asia and North America (Smith & Thiers 1971, Thiers 1975, Both 1993, Zang 1999, Bessette et al. 2000, Binder & Hibbett 2004, Muñoz 2005), which differs in pileus color (predominantly ochre and brown in var. *luridus*; red in var. *rubriceps* (Maire) Dermek; and orange or ochre-orange in var. *queletiformis* J. Blum), red pores, a red reticulum restricted to 2/3 of the stipe, and orange-red flesh that is red-purple only at the stipe base and fades to yellow-olive after exposure. *Boletus luridus,* a typical species from broad-leaved forests in calcareous soils, also differs microscopically in its slightly smaller spores [(12–)12.3–13.9(–14.2) × 5–5.9(–6.4) µm], caulocystidia that are fusiform, utriform, or lageniform, and a pileipellis composed of short end cells (Muñoz 2005).

Boletus luteocupreus has a chrome-yellow pileus (sometimes with pinkish tones) becoming ochre-orange with age, a delayed (a few hours) red-coppery or brown-coppery staining reaction, variably reddish pores, a yellow-orange stipe that is red or red-carmine toward the base and almost completely covered with a blood-red reticulum, an association with deciduous forests in acidic soils, smaller spores [(10.4–)10.9–12.3(–12.9) × 4.5–5.3(–5.9) µm], and fusiform or lageniform caulocystidia (Muñoz 2005).

Boletus rhodopurpureus f. *rhodopurpureus*, known also in Asia (Zang 1999; Binder & Hibbett 2004), is distinguished by a pink, purple to red vinaceous pileus, sporocarps with red pores when young and red-orange in age (except at the yellow-orange margin), a yellow or yellow-orange stipe that is red-orange toward the base with garnet or red purple context only at the base and with a red fine reticulum limited to the apex, and a habit in broad-leaved forests (mainly *Quercus* spp.) in acidic soils. It is differentiated microscopically by smaller spores (11.8–13.6(–14.2) × (4.8–)5.1–5.7(–6) µm), and fusiform caulocystidia (Muñoz 2005).

Among extra-European taxa, *B. atlanticus* is morphologically similar to *B. floridanus* (Singer) Murrill and *B. sinicus* W.F. Chiu.

Boletus floridanus, reported from North and Central America (from North Carolina and Tennessee, south to Florida, Texas, Mexico, and Belize), is characterized by a grayish-red, intense red to vinaceous pileus, yellow tubes with red pores, a red markedly reticulated stipe, pale to bright yellow context, a habit in broad-leaved (especially oak) forests, and longer narrower spores (12.8–18 × 4–5.6 µm) (Both 1993; Bessette et al. 2000, 2007; Ortiz et al. 2007).

Boletus sinicus, described from Yunnan (China), has a fibrillose scaly garnet brown pileus, deep red pores, a concolorous (or apically yellow) stipe reticulated with prominent red veins, white or yellowish flesh, and smaller spores (7.5–11 × 4.5–5.5 μm) (Chiu 1948, 1957; Wang et al. 2004).

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An efficient protocol for DNA extraction from *Meliolales* and the description of *Meliola centellae* sp. nov.

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Abstract — The first black mildew fungus associated with *Apiaceae* is collected from the state of Minas Gerais, Brazil. The new species is described, illustrated, and compared morphologically with species reported on hosts belonging to the related *Araliaceae*. Additionally, two efficient methods for extracting DNA from biotrophic fungi are described. Phylogenetic 28S rDNA sequence analyses confirm *Meliolales* as a distinct order within *Sordariomycetes*.

Key words — *Ascomycetes*, medicinal plant, *Meliolaceae*, tropical fungi, phylogeny

Introduction

Centella asiatica is a native plant of tropical Asia and commonly grows in humid areas in many tropical regions. This plant was introduced in Brazil and grows as a weed in the lawns of gardens in the southern and southeastern regions (Kissmann & Groth 1999). Brazilian researchers have paid relatively little attention to fungi associated with *C. asiatica*. Among the 20 fungal species described on this host worldwide, only three are reported from Brazil: *Capnodium brasiliense* Puttemans, *Septoria asiaticae* Speg., and *Vitalia setofasciculata* Bat. et al. (Farr & Rossman 2012, Mendes & Urben 2012).

During recent surveys of black mildew fungi in Brazil, several species were recorded on representatives of *Anacardiaceae*, *Asteraceae*, *Bignoniaceae*, *Burseraceae*, *Euphorbiaceae*, *Fabaceae* (*Caesalpinioideae* and *Papilionoideae*), *Piperaceae,* and *Sapindaceae* (Pinho et al. 2009, 2012; Macedo et al. 2010; Silva et al. 2012). The present work includes the description of a new species collected on *C. asiatica*.

Black mildew fungi infect plant species from numerous botanical families and are widely distributed in the tropics and subtropics (Hansford 1961, Hosagoudar 1996). As these fungi are strictly obligate biotrophs and must interact with living plant cells for growth and reproduction, they are usually host specific or have a very narrow host range (Hansford 1961; Hosagoudar 1996). Thus, attempts to grow species of *Meliolales* in pure culture have not been successful, making their DNA extraction challenging (Hansford 1961, Hosagoudar 1996, Vitoria et al. 2010). The development of an efficient protocol for DNA extraction from this fungal group would allow the taxonomic value of different morphological characters to be determined and might reveal discriminatory differences previously overlooked (Saenz & Taylor 1999, Lumbsch & Huhndorf 2007, Rodriguez & Piepenbring 2007, Vitoria et al. 2010).

In the present paper we provide a detailed account of the black mildew fungus found on *C. asiatica* and describe it as a new *Meliola* species based on its morphology and purported host specificity. In addition, we present two efficient DNA extraction methods from biotrophic fungi and the phylogenetic relationship of *Meliolales* based on 28S rDNA nucleotide sequences.

Materials & methods

Morphology

Centella asiatica plants were found covered with black colonies on both sides of the leaves during September 2009 in the "Mata da Dendrologia", a fragment of secondary tropical seasonal semi-deciduous montane forest, a component of the Brazilian Atlantic forest in the campus of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. Symptomatic leaves were collected, photographed, and dried in a plant press. Fresh samples examined under an Olympus SZ40 stereomicroscope were promptly recognized as a meliolaceous fungus. Representative structures were either scraped with a scalpel or removed with an adhesive tape and mounted in lactophenol. Slides containing the fungal structures were examined and photographed under an Olympus BX 51 light microscope equipped with an Olympus e-volt 330 digital camera. Illustrations were prepared with a drawing tube and finalized with the method described by Barber & Keane (2007). For scanning electron microscopy, air-dried material was directly mounted and coated by a thin layer of gold in a sputter coater (Balzers® model FDU 010) for 2 min. Photographs were made with a Carl-Zeiss Model LEO VP 1430 scanning electron microscope (SEM). Biometric data was based on 30 measurements of structures. A representative specimen was deposited in the local herbarium at the Universidade Federal de Viçosa (Herbarium VIC).

DNA extraction

To obtain a representative fungal DNA, ca. 50 fertile perithecia of *Asteridiella obesa* (Speg.) Hansf., *Irenopsis heveae* Hansf., *Meliola centellae,* and *M. vernaliae* D.B.Pinho & O.L.Pereira were examined under a stereomicroscope to check for possible contamination by other fungi. They were removed with the aid of a fine glass needle and placed into

a microcentrifuge tube (1.5 ml) containing 5 µl of double distilled water and stored at –20°C for later use. Total genomic DNA was extracted by using Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, U.S.A.) with some adjustments as follows:

Fungal samples were processed by freezing the sample with liquid nitrogen and grinding it into a fine powder using a microcentrifuge tube pestle. The crushing continued after adding 100 µl Nuclei Lysis Solution + 100 mg polyvinylpyrrolidone. After 500 µl of the same solution was added, the sample mixture was vortexed at high speed for 3–5 seconds to wet the tissue. The samples were incubated at 65°C for 15 minutes. After the solution was cooled at –20°C for 5 min, 200 µl Protein Precipitation Solution was added, mixed in a vortex at high speed for 20 seconds, and centrifuged for 10 min at 14000 rpm. The supernatant (about 600 µl) containing the DNA was transferred to a clean 1.5 mL microcentrifuge tube containing 600 µl of cold isopropanol. The solution was mixed by inversion until thread-like DNA strands formed a visible mass and then stored at –20°C for 24 hours. The next day the solution was centrifuged at 14000 rpm for 5 min, and the supernatant was discarded. 600 µl cold ethanol was added to the pellet and centrifuged at 14000 rpm for 5 min. This ethanol step was repeated again to wash the DNA. After the ethanol was removed, the tube was put upside down over clean towel paper and the pellet was air dried for 30 min. As a final step, the pellet was re-suspended in 20 µl DNA Rehydration Solution and 3 µl RNase Solution was added and mixed by inverting the tube, which was incubated at 37°C for 1 hour. The sample was then stored at –20°C for later use.

Direct amplification

For direct amplification of the 28S rDNA, ca. 20 clean (free of mycoparasites) fertile perithecia were removed using a fine glass needle and placed into a 0.2 ml microcentrifuge tube containing 3 µl double distilled water (Jaklitsch & Voglmayr 2012). After soaking in the water, the samples were squashed with a sterile forceps to release their contents and mixed in a vortex at high speed for 1 min. We also tested the protocol from Vitoria et al. (2010), replacing 3 µl double distilled water by 3 µl Cell Lysis Buffer (0.05M NaOH, 0.25% [w/v] SDS). The samples were frozen for 15 min at –20°C and thawed in a water bath at 80°C for 15 min, modifying the protocol in Griffin et al. (2002). This process was repeated three times using crushed dry ice and thawing in a water bath. When the solution cooled, the ingredients for PCR reactions were added.

PCR amplification and DNA sequencing

For each 25 µl PCR reaction we used 12.5 µl Dream Taq TM PCR Master Mix 2X (MBI Fermentas, Vilnius, Lithuania), 1 µl each of 10 µM forward and reverse primers (Invitrogen, Carlsbad, EUA), 1 µl dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, U.S.A.), 5 µl 100× (10 mg/mL) Bovine Serum Albumin (BSA, Sigma-Aldrich, St. Louis, MO, U.S.A.), 2 µl genomic DNA, and nuclease-free water to bring the total volume to 25 µl.

The primers LR0R (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGAGGGAAACTTCG-3') and ITS1 (5'-TCCGTAGGTGAACCTGCGG–3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify the partial 28S rDNA and ITS, respectively (Vilgalys & Hester 1990, White et al. 1990). Amplifications began with an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 53°C for

Table 1. Genbank accession numbers of 28S rDNA sequences derived from strains used in the phylogenetic analysis.

Species	Voucher	28S rDNA
Aphysiostroma stercorarium	ATCC 62321	AF543792
Apiospora bambusae	ICMP 6889	DQ368630
A. setosa	ICMP 4207	DQ368631
Appendiculella lozanellae	MP3432	DQ508302
Asteridiella sp. 1	PPMP 796	EF094839
Asteridiella obesa	VIC31239	JX096809
Balansia henningsiana	AEG 96-27A	AY545727
Camarops petersii	JM1655	AY346265
C. tubulina	SMH4614	AY346266
C. ustulinoides	SMH1988	AY346267
Ceratocystiopsis manitobensis	CW13792	DQ294358
C. minima	CMW162	DQ294361
Ceratocystis fimbriata	CBS 374.83	AF221009
Chaetosphaeria ovoidea	SMH 2605	AF064641
Claviceps paspali	CBS 110.22	U47826
Coniochaeta leucoplaca	JONG 54	FJ167399
C. ostrea	AFTOL-ID 915	DQ470959
C. velutina	UAMH 10912	EU999180
Coniochaetidium savoryi	TRTC 51980	AY346276
Cornipulvina ellipsoides	—	DQ231441
Cryphonectria parasitica	CMW 13749	AF277132
Diaporthe phaseolorum	CBS 435.87	U47830
Diatrype disciformis	CBS 197.49	U47829
Discostroma botan	HHUF 4642	DQ368629
Endomeliola dingleyae	PDD 98304	GU138866
Faurelina elongata	CBS 126.78	DQ368625
Gelasinospora tetrasperma	ATCC 96230	AY346281
Gnomonia ribicola	CBS 115443	DQ368626
Grosmannia grandifoliae	CMW703	DQ294399
G. penicillata	CMW470	DQ294385
Halosarpheia fibrosa	JK5132C	U46886
Halosphaeria appendiculata	CBS 197.60	U46885
Hypocrea pallida	GJS89-83	U00740
H. schweinitzii	CBS 243.63	U47833
Hypomyces polyporinus	CBS 168.89	AF543793
Irenopsis sp.	VIC31752	JX096807
Melanochaeta hemipsila	SMH 2125	AY346292
Meliola centellae	VIC31244	JQ734545
M. variaseta	DRJ 54	EF094840
M. vernaliae	VIC31240	JX096808
Nectria cinnabarina	IAM 14568	AF193237
Neurospora crassa	CBS 709.71	AY681158
Ophiostoma lunatum	CMW10564	DQ294355
O. nigrocarpum	CMW651	DQ294356
O. piliferum	CBS129.32	AY281094

Table 1, concluded

Species	Voucher	28S rDNA
O. ulmi	CBS 298.87	DQ368627
Plagiostoma euphorbiae	CBS 340.78	AF277131
Poroconiochaeta discoidea	SANK 12878	AY346297
Pyxidiophora arvernensis	AFTOL-ID 2197	FJ176894
Seiridium cardinale	ATCC52521	AF382377
Sordaria fimicola	CBS 723.96	AF132330
S. macrospora	ATCC 36709	AY346301
Valetoniellopsis laxa	CBS 191.97	AY015635
Valsa ceratosperma	AR3426	AF408387
Valsonectria pulchella	SMH1193	AY346304
Xylaria acuta	AFTOL-ID 63	AY544676
X. hypoxylon	CBS 499.80	U47841

45s, extension at 72°C for 2 min and a final extension of 7 min at 72°C. PCR products were analyzed on 2% agarose electrophoresis gels stained with GelRed™ (Biotium Inc., Hayward, CA, U.S.A.) in a 1× TAE buffer and visualized under UV light to check for amplification size and purity. PCR products were purified and sequenced by Macrogen Inc., South Korea (http://www.macrogen.com). The nucleotide sequences were edited with the DNA Dragon software (Hepperle 2011). All sequences were checked manually and nucleotides with ambiguous positions were clarified using primer sequences in both directions. Obtained sequences were deposited in GenBank (www.ncbi.nlm.nih.gov).

Phylogenetic analysis

For phylogenetic analysis, 28S rDNA sequences from additional species were retrieved from GenBank (Table 1). Consensus regions were compared against GenBank's database using Mega BLAST program. The closest hit sequences and representatives of selected *Sordariomycetes* (*Boliniales*, *Chaetosphaeriales*, *Coniochaetales*, *Diaporthales*, *Hypocreales*, *Meliolales*, *Microascales*, *Ophiostomatales*, *Sordariales*, and *Xylariales*) were obtained from Genbank (www.ncbi.nlm.gov) to help clarify the phylogenetic relationship of *Meliolales* within the class. All sequences were downloaded in FASTA format and aligned using the multiple sequence alignment program MUSCLE® (Edgar 2004) built in MEGA v. 5 software (Tamura et al. 2011). Alignments were checked and necessary manual adjustments were made. All ambiguously aligned regions within dataset were excluded from the analysis. Gaps (insertions/deletions) were treated as missing data. The resulting alignment was deposited in TreeBASE (http://www.treebase.org/) (accession number S12768).

Bayesian inference (BI) analysis employing a Markov Chain Monte Carlo method (MCMC) was performed. Before launching BI, the best nucleotide substitution models were determined with MrModeltest 2.3 (Posada & Buckley 2004). After calculation of likelihood scores, models were selected according to the Akaike Information Criterion (AIC). The general time-reversible evolution model (Rodriguez et al. 1990) was used, including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G). The alignment was phylogenetically analyzed on CIPRES webportal (Miller et al. 2010) using MrBayes v.3.1.1 (Ronquist & Huelsenbeck

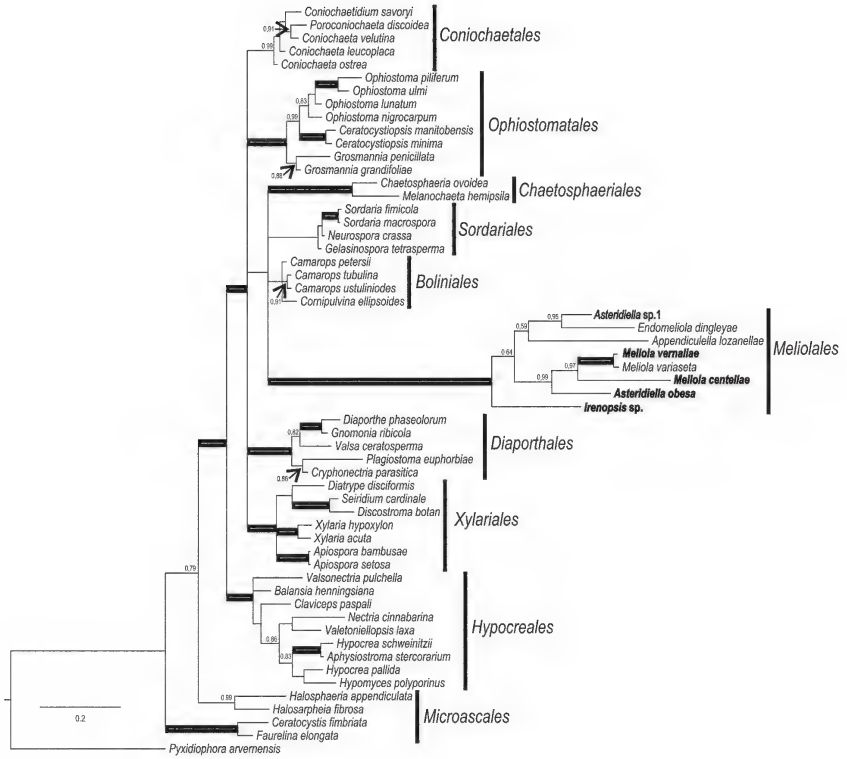


FIG. 1. Phylogenetic tree inferred from Bayesian Analysis of 28S rDNA *Sordariomycetes* sequences. Bayesian posterior probability of >70% are indicated above the nodes and bold lines indicate posterior probabilities of 1.00. The species in study are in bold. The tree is rooted with *Pyxidiophora arvernensis*.

2003). Four MCMC chains were run simultaneously, starting from random trees for 10,000,000 generations. Trees were sampled every 1000th generation for a total of 10,000 trees. The first 2500 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala & Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 7500 trees. Convergence of the log likelihoods was analyzed with TRACER v. 1.4.1 (Rambaut & Drummond 2003); no lack of convergence was detected. Trees were visualized in FigTree (Rambaut 2009) and exported to graphics programs. *Pyxidiophora arvernensis* (Breton & Faurel) N. Lundq. was used as outgroup in these analyses.

Results

Meliola centellae Pinho & O.L. Pereira, **sp. nov.** FIGS 2–3

MYCOBANK MB519037

Differs from *Meliola pectinata* by its smaller ascospores and longer mycelial setae.

TYPE: Brazil, Minas Gerais, Mata da Dendrologia, on living leaves of *Centella asiatica* (L.) Urb. (*Apiaceae*), 16 September 2009, D.B. Pinho (**Holotype**, VIC 31244).

ETYMOLOGY: The epithet refers to the host genus, *Centella*.

BEELI FORMULA 3112.3232. Colonies black, minute, amphigenous, mostly epiphyllous, initially scattered but becoming confluent with age, dense, 1–3 mm diameter. Hyphae dark brown, septate, straight to sub-straight, branching usually opposite at acute angles, bearing appressoria and conidiogenous cells. Hyphal cells 11–21 × 5–9 µm. Appressoria opposite or alternate, antrorse, straight to curved; stalk cells dark brown, cylindrical to cuneate, 2.5–5 × 5–7.5 µm, head

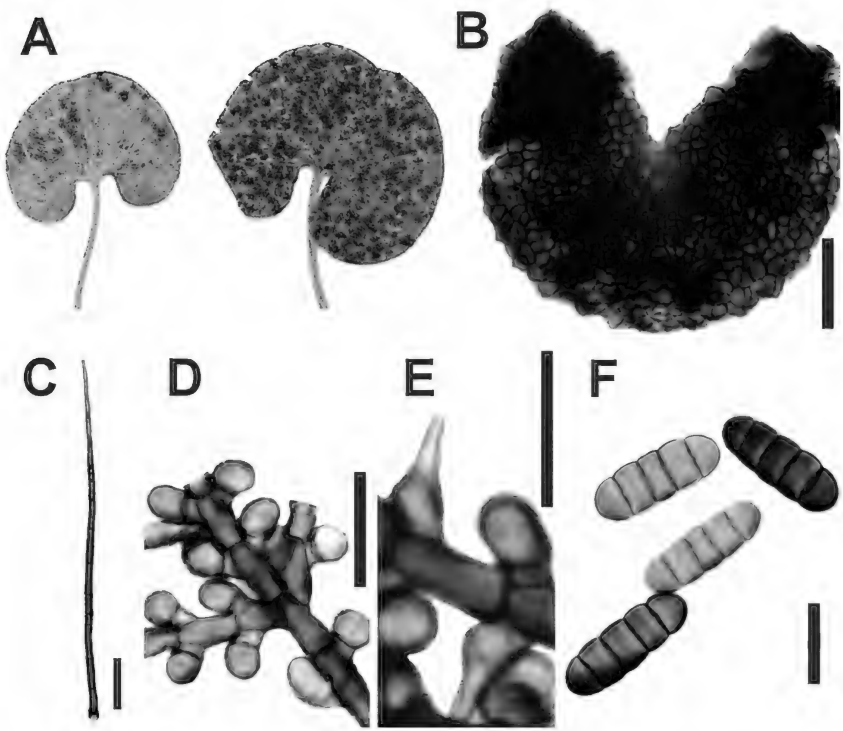


FIG 2. *Meliola centellae* on *Centella asiatica*. A. General view of abaxial (left) and adaxial (right) colonies on leaf surface. B. Perithecium. C. Mycelial setae. D. Appressoria. E. Mixed appressoria and phialides. F. Ascospores. Bars: B, C = 50 µm; D–F = 25 µm.

cells dark brown, globose to ovate, slightly attenuated at the apex, rounded, entire, 7.5–12.5 × 5–10 µm. Phialides light brown, mixed with appressoria, opposite, ampulliform, 12.5–25 × 6–9 µm. Mycelial setae dark brown, simple, straight, apex acute, 5–12 septate, 218–430 × 7.5–10 µm. Perithecia brown, globose, scattered, black, 152–247 µm diameter. Asci evanescent. Ascospores hyaline inside the ascus, becoming grey or brown with age, dark brown or grey at maturity, cylindrical to ellipsoid, rounded at the tips, 4-septate, constricted at the septa, 32–39.5 × 9.5–12.5 µm.

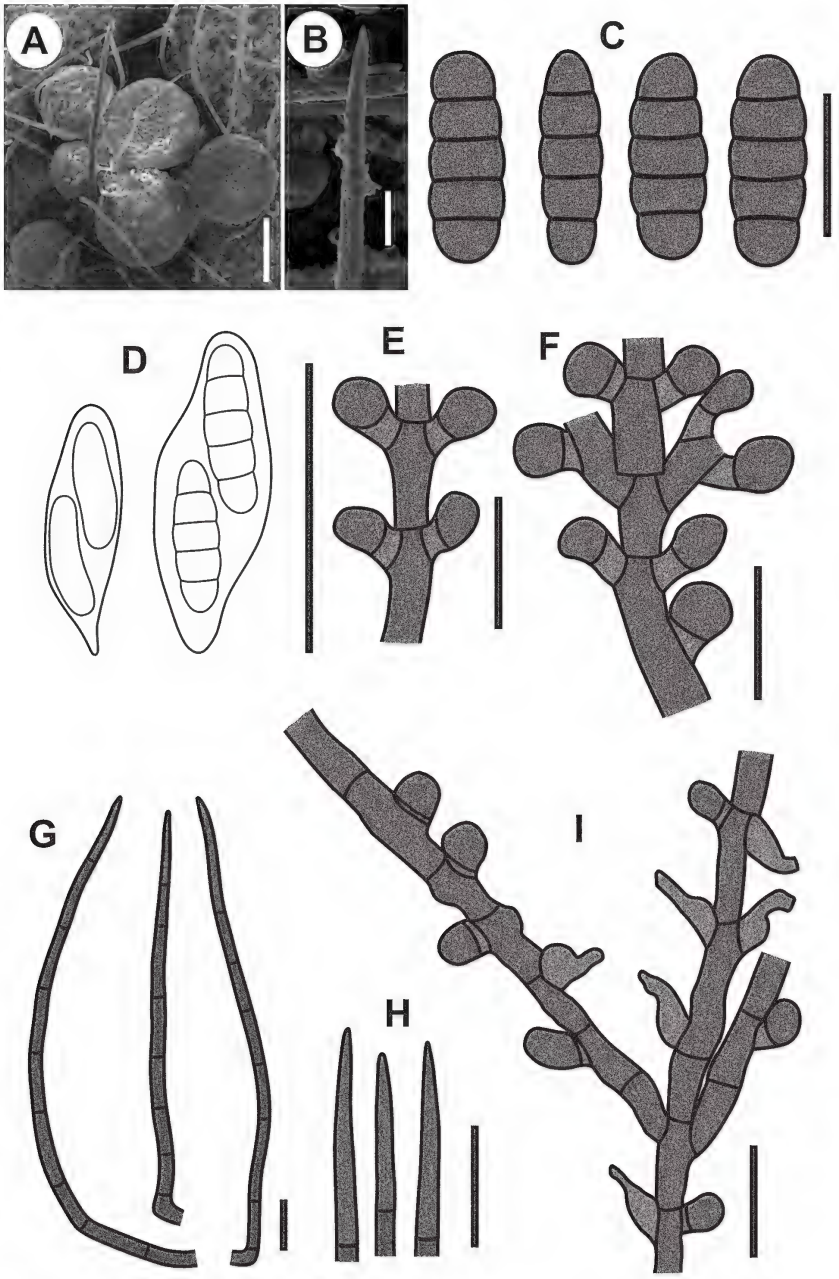
Molecular analyses: The PCR-amplified fragments were obtained by direct amplification and extraction with Wizard® Genomic DNA Purification. Both protocols were found suitable for DNA extraction of meliolaceous fungi. On the other hand, the protocol from Vitoria et al. (2010) was not appropriate for *Meliola* spp. The amplification of the partial 28S rDNA revealed sequences of ca. 700 bp. Although the ITS sequences were not used in phylogenetic analysis, they were lodged in GenBank (Accession No. KC252606–KC252608) for future studies and identification purposes. The manually adjusted alignment contained 57 strains (including the new species described here). Of the 467 characters used in the phylogenetic analysis, 209 were parsimony-informative, 252 were variable, and 207 were conserved. The Bayesian Inference tree confirms *Meliolales* as a distinct order within *Sordariomycetes*.

Discussion

Centella asiatica belongs to *Apiaceae* subfam. *Mackinlayoideae*, which is somewhat intermediate between *Apiaceae* sensu lato and *Araliaceae* (Plunkett et al. 2004, Oskolski & Wyk 2010). Because no other black mildew fungus has been reported on hosts in the *Apiaceae*, we compared the *C. asiatica* fungus with *Meliola* spp. occurring on taxa in *Araliaceae*.

Of the 19 species and four infraspecific taxa of *Meliola* known on *Araliaceae* (Hansford 1961, Hosagoudar et al. 1995, Hosagoudar 1996, Hosagoudar & Archana 2009), none matched *M. centellae*. Six taxa (*M. acanthopanacis* W. Yamam., *M. araliicola* W. Yamam., *M. didymopanacis* var. *stevensii* Hansf., *M. schefflerae* Hansf., *M. payakii* Hosag., and *M. pectinata* Höhn.) have straight simple mycelial setae (like *M. centellae*) but differ in several other traits (Hansford 1961, Hosagoudar 1996). *Meliola pectinata* matches the first four characters of the *M. centellae* Beeli formula but has larger ascospores and shorter mycelial setae. *Meliola acanthopanacis*, *M. araliicola*, *M. didymopanacis*

Fig 3. *Meliola centellae* on *Centella asiatica*. A. SEM photographs of the perithecia and mycelial setae (B). C. Ascospores. D. Asci with two immature ascospores. E, F. Superficial hyphae with two-celled oppositely and alternately positioned lateral appressoria. G. Mycelial setae. H. Detail of mycelial setae with acute apex. I. Superficial hyphae with phialides and appressoria. Bars: A= 100 µm; B= 20 µm; C–I = 30 µm.


A
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var. *stevensii*, and *M. payakii* also have larger ascospores than *M. centellae*. *Meliola centellae* is also very similar to *M. schefflerae* but also produces larger ascospores (45–51 × 17–19 µm; Hansford 1961, Hosagoudar 1996). In addition, the similar *M. heteroseta* Höhn. and *M. dichotoma* var. *kusanoi* (Henn.) Hansf. are distinguished from the new species by larger ascospores and dichotomously branched mycelial setae (Hansford 1961). We therefore propose *Meliola centellae* for the first *Meliola* species (and the first *Meliolaceae*) recorded on a member of the *Apiaceae*.

Although mycologists in several laboratories tried to isolate DNA from meliolalean fungi, only a few attempts have been successful (Rodriguez & Piepenbring 2007). The *Meliolales* are a non-culturable group frequently contaminated by numerous other fungi. It is thought that the dark pigment of their thick cell walls might also interfere with DNA isolation (Rodriguez & Piepenbring 2007). Although the Vitoria et al. (2010) method has been reported as suitable for extracting DNA from ascomata, we found it inappropriate for *Meliola* spp. Here we successfully extracted DNA from *Meliolales* through direct amplification and extraction with Wizard® Genomic DNA Purification kit. Successful direct amplification of fungal DNA have been published previously for *Xylariales* and *Meliolales* (Saenz & Taylor 1999, Rodriguez & Piepenbring 2007, Jaklitsch & Voglmayr 2012), but adding the freezing/thawing steps probably improved the process. This is commonly used for most species that possess rigid cell walls and resist lysis techniques by compromising the integrity of the cell wall (Griffin et al. 2002). The extraction with Wizard® Genomic DNA Purification kit is described herein as the first successful protocol for isolating and purifying DNA from *Meliolales*. This procedure allows amplification of multiple gene regions.

Of the several papers revising the systematics of the *Sordariomycetes* (Spatafora et al. 2006, Zhang et al. 2006, Lumbsch & Huhndorf 2007, Tang et al. 2007, Schoch et al. 2009), only Saenz & Taylor (1999) included members of the *Meliolales*. Their phylogeny grouped *Meliolales* in a distinct clade close to *Sordariales* and thus showed that phylogenetic analysis of molecular characters confirmed previous morphologically based classifications placing *Meliolales* in the *Sordariomycetes*. The most widely used gene sequences for phylogenetic studies have been 28S rDNA genes because of their easy amplification due their high copy number and the availability of numerous universal primers (Miller & Huhndorf 2005, Tang et al. 2007). The present paper confirms that *Meliolales* comprises a monophyletic order within *Sordariomycetes* (Fig. 1). Although the presence of dark perithecia and inamyloid asci indicate that *Meliolales* belong to the subclass *Sordariomycetidae* (Zhang et al. 2006), the phylogenetic position of the order is still uncertain because the phylogeny inferred from the 28S rDNA datasets are the least supported (Tang et al. 2007). Miller & Huhndorf

(2005) propose that increased taxon sampling will improve resolution in many clades and that phylogenetic support will increase through the incorporation of additional genes. This clearly indicates the need for extensive taxon sampling and further investigation of DNA sequences in order to better clarify the phylogeny of the *Meliolales*.

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Tuber sinosphaerosporum sp. nov. from China

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Abstract — A new white truffle species from China is described and illustrated. *Tuber sinosphaerosporum* is characterized by its white ascomata and globose ascospores ornamented with large reticulum meshes. The new species, supported by ITS sequence analysis, offers commercial value due to its moderate to relatively large size and possibly high yield in China.

Key words —*Ascomycota*, taxonomy

Introduction

The number of *Tuber* species in China has increased considerably over the last thirty years (Liu 1985, Wang 1988, Wang & Li 1991, Wang et al. 1998, Wang & He 2002, Tao et al. 1989, Hu 1992, Xu 1999, He et al. 2004, Hu & Wang 2005, Chen et al. 2005, Chen & Liu 2007, Cao 2010, Fan et al. 2011, 2012a,b), and new species are still being found. In December 2010, about a kilo of fresh white truffles growing under *Corylus* sp. was collected from Baoshan City of Yunnan Province; they had a strong but pleasant aroma of garlic, typical of white truffles. Subsequently, another white truffle was collected from Chenggong County near Kunming City of Yunnan that was morphologically similar to the Baoshan specimens but grew under conifers. Detailed morphological observation and molecular research confirm that the collections from both locations represent a single undescribed species.

Materials & methods

Morphological studies

Truffles were collected from Baoshan City and Chenggong County, Yunnan Province. Macroscopic characters were described from fresh specimens. Microscopic characters were described from razor-blade sections of fresh specimens mounted in 3% KOH,

Table 1 *Tuber* specimens and sequence numbers used in molecular studies.

Species name	Voucher	Origin	ITS	Reference
T. borchii Vittad.	GB62	Italy	HM485342	Bonito et al. 2010
	GB39	Unknown	HM485343	Bonito et al. 2010
	GB45	Italy	HM485344	Bonito et al. 2010
T. borchii var. *sphaerospermum* Malençon	HKAS 520005	China	GQ 217541	GenBank
T. californicum Harkn.	L4AB7	Unknown	EF411102	Morris et al. 2008
	JT22590	USA	HM485351	Bonito et al. 2010
	JT28058	USA	HM485346	Bonito et al. 2010
T. latisporum Juan Chen & P.G. Liu	HKAS 30838B	China	DQ898185	Chen & Liu 2007
	HKAS42380	China	DQ898184	Chen & Liu 2007
	HKAS44315	China	DQ898183	Chen & Liu 2007
T. melanosporum Vittad.	A59	France	AF106878	From GenBank
	TM13	France	AF132501	Roux et al. 1999
T. oligospermum (Tul. & C. Tul.) Trappe	MA: FUNGI: 41010A	Spain	FM205506	GenBank
	MA: FUNGI: 41010B	Spain	FM205507	GenBank
	MA: FUNGI: 28388B	Spain	FM205508	GenBank
	MA: FUNGI: 28389	Spain	FM205509	GenBank
T. puberulum Berk. & Broome	TL11885	Denmark	AJ969626	Tedersoo et al. 2006
	TL3857	Denmark	AJ969625	Tedersoo et al. 2006
	BI-32	Hungary	AJ557537	Halász et al. 2005
T. sinosphaerosporum	BJTC FAN135 (HOLOTYPE)	China	JX092086	This study
	BJTC FAN136	China	JX092087	This study
T. sphaerosporum Gilkey	JT12487	USA	FJ809853	Bonito et al. 2010
	JT19772	USA	FJ809854	Bonito et al. 2010
	JT12487	USA	GQ221449	From GenBank
	OSC75864	USA	HM485390	From GenBank

Melzer's reagent, or 0.1% (w/v) cotton blue in lactic acid. The specimens are deposited in BJTC (Biology Department Herbarium, Capital Normal University). For scanning electron microscopy (SEM), spores were scraped from the dried gleba, placed on to doubled-sided tape, mounted directly on an SEM stub, coated with gold–palladium, and then examined and photographed with a HITACHI S-4800 SEM.

Molecular methods

Herbarium samples were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 ml tube together with one 3 mm diameter tungsten carbide ball. Total genomic DNA was then extracted using the PeqLabE.Z.N.A._Fungal DNA kit following the manufacturer's protocol. The ITS region was amplified with PCR using the primers ITS1/ITS4 (White et al. 1990). PCR was performed in 50 µl reactions containing DNA template 2 µl, primer (10µM) 2 µl each, 2 × Master Mix (Tiangen Biotech (Beijing) Co. Ltd.) 25 µl. PCR reactions were run as follows: an initial

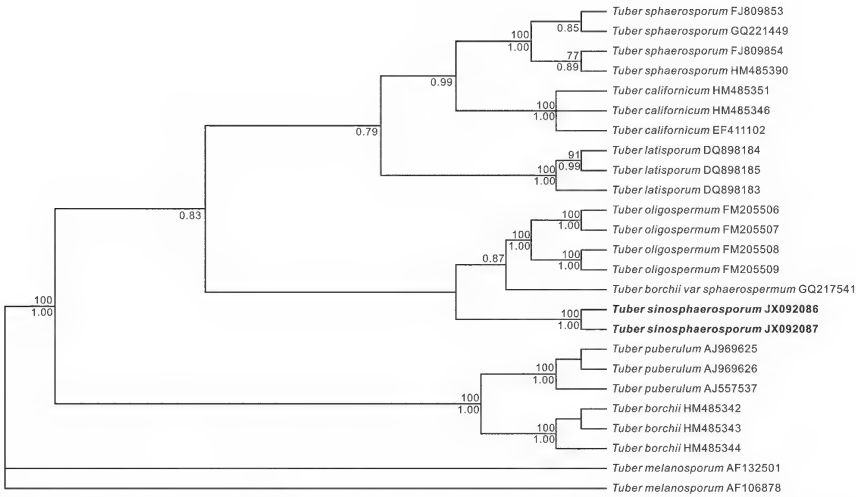

FIG. 1. Phylogeny derived from maximum parsimony ITS rDNA sequence analysis of some *Tuber* species with reticulate ascospore ornamentation using *T. melanosporum* as outgroup. Bootstrap values of >70% from 1000 replications are shown above the respective branches. Clades with Bayesian posterior probabilities (PP) estimated >0.70 (70%) are marked under the branches.

denaturation at 95°C for 3 min, followed by 30 cycles at 95°C for 2 min, 55°C for 25 s, 72°C for 2 min, and a final extension at 72°C for 10 min. The PCR products were sent to Invitrogen Biotechnology Co. Ltd. (Beijing, China) for purifying, sequencing, and editing. The other sequence data of ITS rDNA included in this study were downloaded from GenBank. GenBank numbers are shown in TABLE 1.

Phylogenetic analyses

DNA sequences were aligned with Clustal X (Thompson et al. 1997). The alignment was manually adjusted with Se-Al v.2.03a (Rambaut 2000). The aligned dataset was analyzed with maximum parsimony (MP) using PAUP*4.0b10 (Swofford 2002). Maximum parsimony analysis was conducted using heuristic searches with 1000 replicates of random-addition sequence, tree bisection reconnection (TBR) branch swapping algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap (BS) analysis was performed with 1000 replicates, each with 10 random taxon addition sequences. TBR branch swapping was employed. The Bayesian analysis was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003) with two sets of four chains (one cold and three heated) and the stoprule option in effect, halting the analyses at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100, and the first 25% trees were removed as burn-in. Bayesian posterior probabilities (PP) were obtained from the 50% majority rule consensus of the remaining trees. Two sequences derived from *Tuber melanosporum* were used as outgroup.

Results

Molecular phylogenetics

279 of 612 characters were found to be parsimony-informative. Maximum parsimony analysis resulted in one most parsimonious tree (Fig.1) with a length (TL) of 510 steps, consistency index (CI) of 0.7804, retention index (RI) of 0.8948 and rescaled consistency index (RCI) of 0.6983 (for all sites).

The ITS sequence phylogeny (Fig. 1) revealed that sequences from the two Yunnan collections (*Tuber sinosphaerosporum*) were the same and grouped in a clade with strong support (BS = 100, PP = 1.00). They appear to form a sister relationship with *T. borchii* and *T. oligospermum* but with very low support.

Taxonomy

Tuber sinosphaerosporum L. Fan, J.Z. Cao & Yu Li, **sp. nov.** Fig. 2

MycoBank MB800677

Differs from other *Tuber* species in its fresh white ascomata and regular globose ascospores covered by a reticulate ornamentation that is 2–4 meshes across the spore width.

Type: China. Yunnan Province, Baoshan City, under the soil near *Corylus* sp. 11 Dec. 2010, De-fu Liu (**Holotype**, BJTC FAN135; GenBank, JX092086))

Etymology: *sinosphaerosporum* (Lat.), referring to a Chinese species resembling the American *Tuber sphaerosporum*.

Ascomata irregularly globose or lobed, often convolute and with several deep furrows, 1.5–5.5 cm in diam., white or whitish-cream or pale yellow white when fresh, yellow whitish or light yellow brown after dried, the surface poorly puberulent at least at furrows. Odor faint when young, but of strong garlic when mature. Peridium 250–300 μm thick, with two layers: outer layer 100–150 μm thick, pseudoparenchymatous, composed of subglobose and angular cells 7.5–17.5 μm in diam., with slightly thickened walls, yellowish-brown towards the surface; inner layer 150–200 μm thick, texture intricate, the hyphae thin-walled, hyaline, 2.5–5 μm in diam. Hairs hypha-like, arising from the superficial cells in places, 10–40 × 2.5–5(–7.5) μm, hyaline or yellowish, usually thin-walled, 1–2 septate, cylindric and obtuse at the apex. Gleba white at first, becoming yellow-brown to brown at maturity, marbled with large and rare, branched, white veins originating from various points of the inner peridium. Asci globose, subglobose or broadly ellipsoid, sessile, 75–125 × 62.5–85 μm, 1–4-spored. Ascospores regularly globose, hyaline at first, becoming yellow brown to brown at maturity, ornamentation regular reticulum, 20–42.5 μm in 2–4-spored asci and 40–45 μm in 1-spored asci in diam. excluding the ornamentation, the meshes large and variable in size, 5.0–7.5 μm high, usually 2–4 meshes across the spore width.

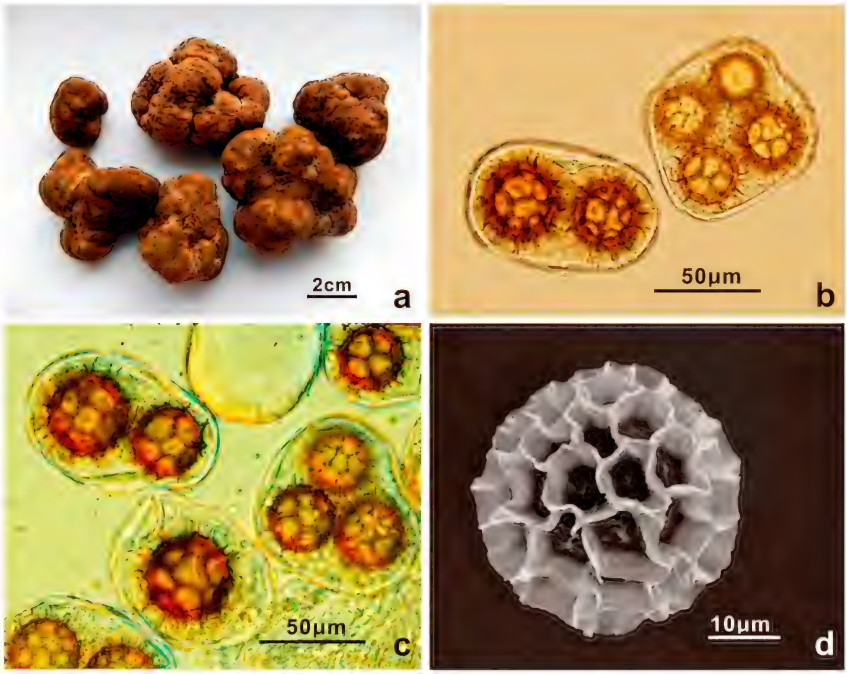

Fig. 2. *Tuber sinosphaerosporum* (BJTC FAN135, holotype). a. Soil adhering to the ascomal surface makes them appear brown; b–c. Asci and ascospores observed under light microscope; d. Ascospore observed under SEM.

Additional specimens examined: China, Yunnan Province, Chenggong County, under soil of conifers, 18 Dec. 2010, Jin-zhong Cao (BJTC FAN136; GenBank, JX092087).

Comments — *Tuber sinosphaerosporum* is similar to American *T. sphaerosporum* in the appearance of ascospores, but the American species differs in its glabrous brown ascomata and dark gleba (Gilkey 1939, 1954). The peridium of *T. sphaerosporum* is composed of "variable cells, the large and small intermixed, somewhat pseudoparenchymatous or often prosenchymatous" (Gilkey 1954), whereas *T. sinosphaerosporum* has typical pseudoparenchymatous peridium. The molecular study (Fig. 1) also demonstrated a considerable separation.

The other *Tuber* species with regular globose ascospores, such as *T. californicum* from North America, *Tuber borchii* var. *sphaerospermum* from Europe, and *T. oligospermum* from Europe and North Africa, are distinguished from *T. sinosphaerosporum* by their reticulum meshes numbering 5–7 across the ascospore width (Gilkey 1939, 1954, Riousset et al. 2001). Phylogenetic analyses (Fig. 1) support *T. sinosphaerosporum* as a distinct species. The

T. sinosphaerosporum sequences form a sister relationship with *T. borchii* and *T. oligospermum,* but the very low support value indicates they are closely related but distinct.

Tuber sinosphaerosporum could be a potential commercial species in the international white truffle market because of its significant larger ascocarp size (medium to relatively large) and strong but pleasant aroma. According to the local people, *T. sinosphaerosporum* could be expected to have a high cropping yield in Yunnan Province.

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Terriera angularis sp. nov. on *Illicium simonsii* from China

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Abstract — *Terriera angularis*, a new species of *Terriera* that develops on leaves of *Illicium simonsii*, was collected from Shennongjia forestry region of Hubei Province, China. Description, illustration, and comments are given for this fungus. The type collection is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — *Rhytismatales*, morphological character, taxonomy

Introduction

Eriksson (1970) erected the segregate monotypic genus *Terriera* B. Erikss. for the type species *T. cladophila* (Lév.) B. Erikss. (syn. *Hysterium cladophilum* Lév.), which develops on twigs of *Vaccinium myrtillus* L. Johnston (2001), who monographed the monocotyledon-inhabiting *Lophodermium* species, divided *Lophodermium* into five groups, equating group B with *Terriera* because its morphology was closer to *T. cladophila* than to the type species of *Lophodermium*. Of the 24 species included in *Terriera* (MycoBank, www.mycobank.org/), seven have been reported from China: *T. camelliae*, *T. coacervata*, *T. huangshanensis*, *T. illiciicola*, *T. petrakii*, *T. rotundata*, and *T. simplex* (Chen et al. 2012, Gao et al. 2012, Song et al. 2012, Yang et al. 2011, Zheng et al. 2012).

Recently, during investigations of the *Rhytismatales* from Shennongjia forestry region of Hubei Province, China, a new *Terriera* species was discovered.

Materials & methods

Mature and immature ascomata were selected from the collected specimen. Macroscopic features were observed under a hand lens and stereoscope. Reference collection material was rehydrated in water for 15 min, and 10–15 μm thick fruitbody sections were cut by a freezing microtome (YD-1508-III, China). For observing ascomatal

outlines in vertical section, sections were mounted in lactic acid or cotton blue with pretreatment in water. The colors of the various structures and ascospore contents were observed in water. Gelatinous sheaths surrounding ascospores and paraphyses were examined in water or 0.1% (w/v) cotton blue in lactic acid. Measurements were made from material mounted in 5% KOH or Melzer's reagent and from 30 asci, ascospores, and paraphyses for each specimen. Line and point integrated illustrations of external shapes and internal structures of fruit bodies were drawn using a microscopic drawing tube (Panasoianic XSJ-2, Japan).

Taxonomy

Terriera angularis Y.R. Lin, F. Zhou & Xiao Y. Wang, **sp. nov.** FIGS 1–5

MycoBank MB800350

Differs from *Terriera minor* by its triangular to quadrangular, rarely elliptical, mature ascomata, and its narrower ascospores not quite tapering to both ends.

TYPE: China, Hubei, Shennongjia forestry region, Guanmenshan, alt. ca 1300 m, on leaves of *Illicium simonsii* Maxim. (*Schisandraceae*), 9 July 2010, G.J. Jia & Y.R. Lin 2511 (**Holotype**, AAUF 68619).

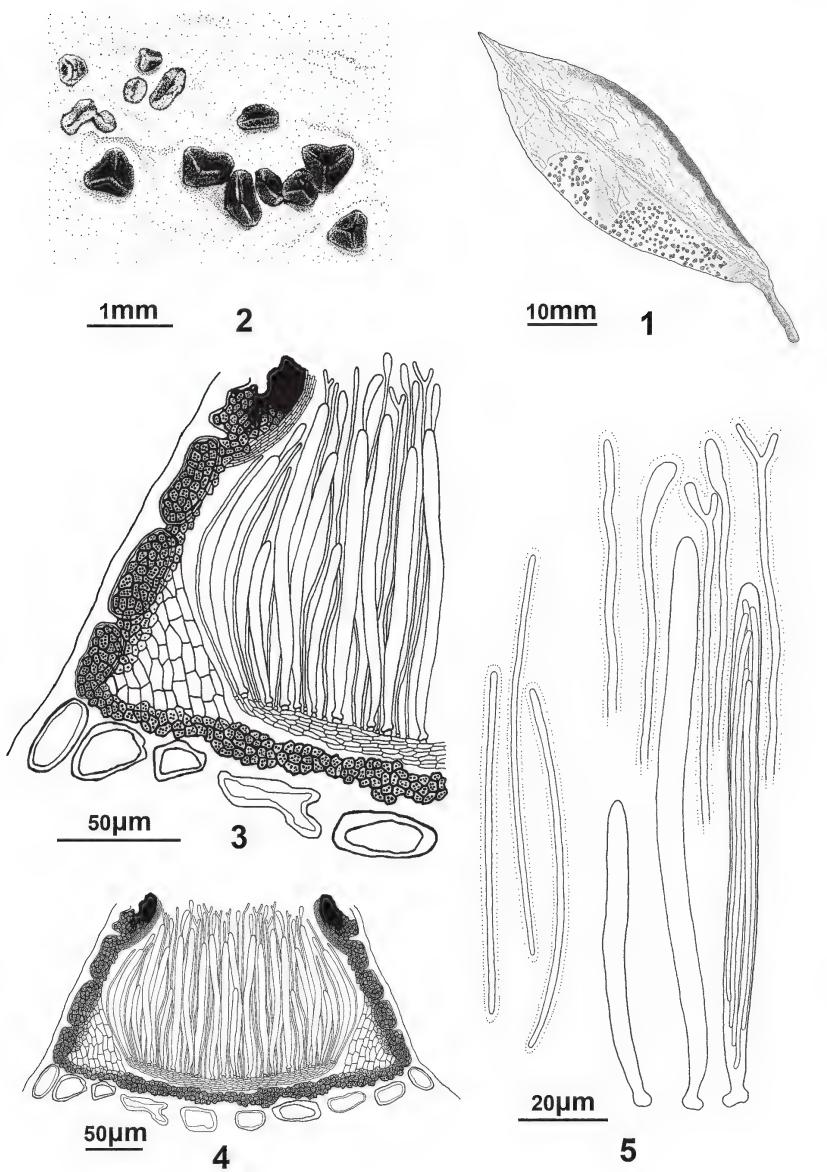
ETYMOLOGY: *angularis* (Latin) = angular, referring to the external shape of the ascomata.

COLONIES only hypophyllous, forming irregular, large bleached spots each with an obvious edge.

ZONE LINES absent.

CONIDIOMATA not observed.

ASCOMATA hypophyllous, clustered, sometimes confluent in groups of two or three. In surface view, ascomata triangular to quadrangular, rarely elliptical, diameter or length 350–720 µm, the edge defined, grey-black to black except sometimes for a paler region at ends, with conspicuously black perimeter line, shiny, moderately raised above the surface of the substratum, opening by 3–4 radial splits or a longitudinal split. Young ascomata almost concolorous with substratum surface, with dark brown perimeter line, usually rotund or elliptical, preformed opening mechanism often appearing as a longitudinally hollow zone. In median vertical section, ascomata subepidermal with epidermal cells becoming filled with fungal tissue as ascoma develops. Lips not observed. COVERING STROMA 22–30 µm thick, connecting to the basal stroma, consisting of dark brown textura angularis with thick-walled cells 3–6 µm diam. A short, ca 12 µm thick extension, comprised of strongly black and brittle tissue with no obvious cellular structure, adjacent to the top of the covering stroma. EXCIPULUM very poorly developed, arising from the inner layer of the covering stroma. BASAL STROMA slightly concave, 10–22 µm thick, dark brown, composed of 2–4 rows of 3.5–7 µm diam., angular, thick-walled cells. Colorless to light grey-brown textura prismatica 35–45 µm thick existing between the covering and basal stromata. SUBHYMENIUM 12–20 µm thick, consisting of



Figs 1–5. *Terriera angularis* on *Illicium simonsii*. 1. Habit on leaf. 2. Detail of ascomata, immature ascomata (top left). 3. Portion of ascoma in median vertical section. 4. Ascoma in median vertical section. 5. Paraphyses, asci and ascospores.

textura angularis-porrecta. Paraphyses filiform, 1.8–2 μm wide, aseptate, gradually or suddenly swollen to 4 μm or branching once at the apex, covered with a ca 1 μm thick mucous coating. Asci maturing sequentially, cylindrical, the apex round or slightly acute, 105–130 × 5.5–6.5 μm, thin-walled, J–, 8-spored, with spores arranged in a fascicle. Ascospores filiform, 70–90 × 1–1.2 μm, hyaline, aseptate, not quite tapering to the round base, enveloped in a 0.8–1 μm thick gelatinous sheath.

Ecology & distribution: Ascomata on fallen leaves of *Illicium simonsii*. Known only from the type locality, Hubei, China.

Comments—*Terriera angularis* is very similar to *T. minor* (Tehon) P.R. Johnst. in the way ascomata are embedded and in ascal shape and size. However, *T. minor* produces oblong to oblong-elliptic ascomata with a black flattened area lining both sides of the single longitudinal slit-like opening, wider ascospores (1.5–2 μm) tapering slightly to both ends, paraphyses branching 2–3 times in upper 30–40 μm, and a faint to well-developed paler zone along the future line of the immature ascoma opening (Johnston 1988, 1989a, b).

Terriera cladophila is easily distinguished from the new species by its subcuticular circular to elliptical ascomata, subhymenium consisting of textura angularis and textura intricata, wider asci (6–9 μm), and brown diffuse zone lines (Minter 1996).

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A new species of *Mycena* sect. *Sacchariferae* from the Iberian cushion-shaped *Genisteae*

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Abstract— *Mycena acanthophila*, collected from several localities in Spain, is described as a new species. It specialises by growing on dead branches of two spiny, cushion-shaped species of *Leguminosae*. A complete description, drawings, and microphotographs showing the morphological characters are accompanied by comparisons with morphologically related taxa.

Key words— acanthocysts, *Echinospartum*, *Erinacea*, nomenclature, taxonomy

Introduction

Mycena sect. *Sacchariferae* was first published as a nomen nudum by Kühner (1938) and later validated by Singer (1962). This section is characterised by the presence of acanthocysts in the pileipellis, which form a subhymeniform layer on primordials and remain as terminal hyphal cells of the hyphae when the pileipellis becomes a cutis with age. Desjardin (1995) subdivided sect. *Sacchariferae* into three provisional stirps, *Amparoina*, *Adscendens*, and *Alphitophora*), based on the presence or absence of cherocytes, caulocystidial morphology, and the presence or absence of a basal disc.

Maas Geesteranus & de Meijer (1998) added another stirps named *Fuscinea*, close to *Alphitophora* but with brownish contents in the acanthocysts of the pileipellis.

According to Robich (2003), only seven species of sect. *Sacchariferae* have been found in Europe: *M. adscendens* Maas Geest., *M. alphitophora* (Berk.) Sacc., *M. cecidiophila* A.P. Berg et al., *M. corynephora* Maas Geest., *M. nucicola* Huijsman, *M. occulta* Harmaja, and *M. querciramuli* Robich. Here, we describe an apparently common new species from the Iberian Peninsula with a very

specific habitat: dead branches of *Echinospartum* and *Erinacea*, two spiny cushion-shaped genera in tribe *Genisteae*.

Materials & methods

Specimens were collected in the field and photographed when fresh with a Canon EOS 50D. The macromorphological study was based on both fresh specimens and photographs. The micromorphological study was performed mostly on air-dried basidiomata, rehydrated with commercial ammonia or 3% KOH, and stained with Congo Red. Melzer's reagent was used for testing amyloid and dextrinoid reactions. Measurements were all taken in ammonia solution with a Jeulin light microscope, under a 100× immersion oil objective and 10× oculars. The hilar appendix was excluded for basidiospore measurements, as well as excrescences for the other elements. The range of dimensions outside the parentheses is provided after manually rejecting 5% of the highest and lowest values, while the extremes are provided between parentheses. The Q coefficient is calculated as length/breadth. Drawings were done by direct observation. Dehydrated samples with absolute ethanol were dried by the critical point technique for the scanning electron microscopy (SEM) study, which was done with a Hitachi S-4100 microscope. GPS data are according to the World Geodetic System datum, WGS84. Studied specimens are deposited in the herbaria AH (Universidad de Alcalá, Alcalá de Henares, Madrid), and VAL (Faculty of Biological Sciences, Universitat de València, Valencia), which acronyms follow Thiers (2012). Terminology follows that of Desjardin (1995).

Mycena acanthophila J.C. Zamora & Català, **sp. nov.** PLATES 1–3

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Differs from other species in *Mycena* sect. *Sacchariferae* by its yellowish basidiomata, pliciform lamellae, and clamped hyphae.

TYPE: Spain, Aragón, Huesca, Jaca, San Juan de la Peña, 42°30′18″N 0°38′13″W, alt. 1125 m, on *Echinospartum horridum* branches, 11-X-2010, J. Hernanz, J.C. Zamora & S. Català (**holotypus**, AH 40678; **isotypus**, VAL_MYCO 939).

ETYMOLOGY: From the Greek ἀκανθος, spine, and φίλος, friend, due to the habitat and association with *Echinospartum* and *Erinacea*.

MACROCHARACTERS— PILEUS broadly conical to hemispherical at first, soon broadly convex and with or without an umbo, finally plane or with the margin slightly recurved in over-mature basidiomata; 0.4–1.7 mm diam.; granulose; bright yellow in young basidiomata, pale yellow with a more coloured disc when mature. STIPE central, filiform, cylindrical to slightly bulbous but never with a basal disc; 0.5–4 × 0.08–0.16 mm; dry, with a granulose surface; bright yellow when young, paler when mature. LAMELLAE often present in medium-sized to large basidiomata, adnate to more or less decurrent, 0–6, pliciform, never reaching the margin, pale yellowish to almost white; lamellar edge concolorous with faces, smallest basidiomata often with a smooth hymenophore; lamellulae absent in all cases. SMELL absent. TASTE not recorded.

Plate 1—*Mycena acanthophila* macroscopic features. A, some basidiomata of the holotype collection. B, basidioma showing well-developed lamellae. C, detail of the granulose pileus surface, due to the presence of acanthocysts. D, basidioma with almost smooth hymenophore. (Photographers, A: S. Català; B, C & D: J. Hernanz)

Microcharacters— Basidiospores subglobose to broadly ellipsoid, rarely globose or ellipsoid, often subamygdaliform to citriform (both ends slightly pointed), (7.5–)8–10 × (6–)6.5–8 µm, Q= 1.06–1.35(–1.42), amyloid; hilar appendix about 0.5 µm long. Basidia 4-spored (rarely a few 2-spored), mostly 22–31 × 9.5–11.5 µm, but sometimes a low percentage of basidia with a root-like base, then up to 40 µm long; sterigmata 3.5–5 µm long. Cheilocystidia present in basidiomata with more or less developed lamellae, claviform, 13.5–22 × 8.5–12 µm, covered with 1–3 µm long excrescences in the upper half. Pleurocystidia not seen. Lamellar trama hyphae 2–6 µm wide. Pileipellis composed of 5–8.5 µm wide hyphae and inflated cells ≤11 µm wide with excrescences on the upper side; acanthocysts protruding among hyphae, broadly clavate to sphaeropedunculate, 12–25 × 8–15 µm, covered with 1–3.5 (–4.5) µm long excrescences. Pileitrama hyphae 2.5–8 µm wide (broader near the margin). Caulocystidia claviform, short- to long-stalked, 12–40 × 6.5–12.5 µm, densely covered with 1–3.5(–5) µm long excrescences; sometimes up to 120 µm long, thin- to somewhat thick-walled, smooth hairs appearing at the base, inconstant. Stipitipellis hyphae 1.5–5 µm, densely covered with

1–3 µm long excrescences. Stipititrama hyphae 2.5–10 µm wide. Pileus and lamellar context more or less dextrinoid, stipe context strongly dextrinoid. Clamp connections present in most structures, but often inconspicuous, best seen in subhymenium.

Ecology & distribution—The ecology and distribution of *M. acanthophila* are linked with those of its *Echinospartum* and *Erinacea* hosts. Cushion-shaped communities of *Echinospartum horridum* (Vahl) Rothm. and *Erinacea anthyllis* Link dominate high level mountains on calcareous soils and are subject to some drought. *Echinospartum horridum* is an endemic species from northern Iberian Peninsula to southern France, while *Erinacea anthyllis* is widely distributed in the Iberian Peninsula, southern France, and northern Africa (Talavera 1999). Both hosts are able to grow under hard conditions, like strong winds, drought, poorly developed soils, and cold (Bonet et al. 2009).

Despite these stressful conditions, the microclimate created inside the cushion is suitable for basidiomata development, where a few fungal species can fruit. From an ecological standpoint, the new species can be considered highly specialised. We observed that *M. acanthophila* grows only inside cushions, and we never found it on the most exposed dead branches. What is more, basidiomata quickly dry up when extracted from the cushion when humidity is not extremely high. The species is very common in its habitat, so future records are to be expected in any place where the hosts are present and may even associate with other species of spiny pulvinular *Genisteae*.

Additional specimens examined— **SPAIN**. **Aragón**: Huesca, Arguis, monte Peiró, 42°20′N 1°42′W, alt. 1500 m, on *Echinospartum horridum* branches, 16-X-2010, J. Hernanz (AH 40679); Huesca, Caldearenas, puerto de Monrepós, 42°20′36″N 0°23′38″W, alt. 1280 m, on *E. horridum* branches, 12-X-2010, J.C. Zamora, J.C. Campos & S. Català (AH 40680); Castiello de Jaca, pista de las Blancas, 42°39′03″N 0°34′25″W, alt. 1145 m, on *E. horridum* branches, 11-X-2010, J. Hernanz, J.C. Zamora & S. Català (AH 40681); Fanlo, 42°35′28″N 0°01′17″W, alt. 1380 m, on *E. horridum* branches, 12-XII-2010, J. Hernanz (AH 40682); Jaca, Peña de Oroel, 42°31′N 0°31′W, alt. 1670 m, on *E. horridum* branches, 14-X-2010, J. Hernanz (AH 40683); Jaca, carretera de Navasa a Oroel, 42°31′16″N 0°28′55″W alt. 1060 m, on *E. horridum* branches, 11-X-2010, J. Hernanz, J.C. Zamora & S. Català (AH 40684); Jaca, San Juan de la Peña, 42°30′25″N 0°39′01″W, alt. 1250 m, on *E. horridum* branches, 10-X-2010, J.C. Zamora, S. Català, J. Hernanz & P.P. Daniëls (AH 40685); 13-XII-2010, J. Hernanz, AH 40686; 42°30′N 0°40′W, alt. 1260 m, on *E. horridum* branches, 12-X-2010, J. Hernanz (AH 40687); 42°30′40″N 0°39′47″W, alt. 1275 m, on *E. horridum* branches, 11-XII-2010, J. Hernanz (AH 40688); 42°30′41.5″N 0°39′51.2″W, alt. 1270 m, 28-XII-2010, J. Hernanz (AH 40689); 42°30′18″N 0°41′04″W, alt. 1330 m, on *E. horridum* branches, 13-XII-2010, J. Hernanz (AH 40690); Santa Cruz de la Serós, San Juan de la Peña, 42°30′54″N 0°41′10″W, alt. 1118 m, on *E. horridum* branches, 13-XII-2010, J. Hernanz (AH 40691); Torla, puerto de Cotefablo, 42°36′56″N 0°12′07″W, alt. 1510 m, on *E. horridum* branches, 12-XII-2010, J. Hernanz (AH 40692); Villanúa, fuente del Paco, 42°04′13″N 0°03′13″W, alt. 1190 m, on *E. horridum* branches, 10-X-2010, J.C. Zamora & S. Català (AH 40693); Yebra de Basa, Santa Orosia, 42°30′N 0°15′W, alt. 1530 m, on *E. horridum* branches, 15-

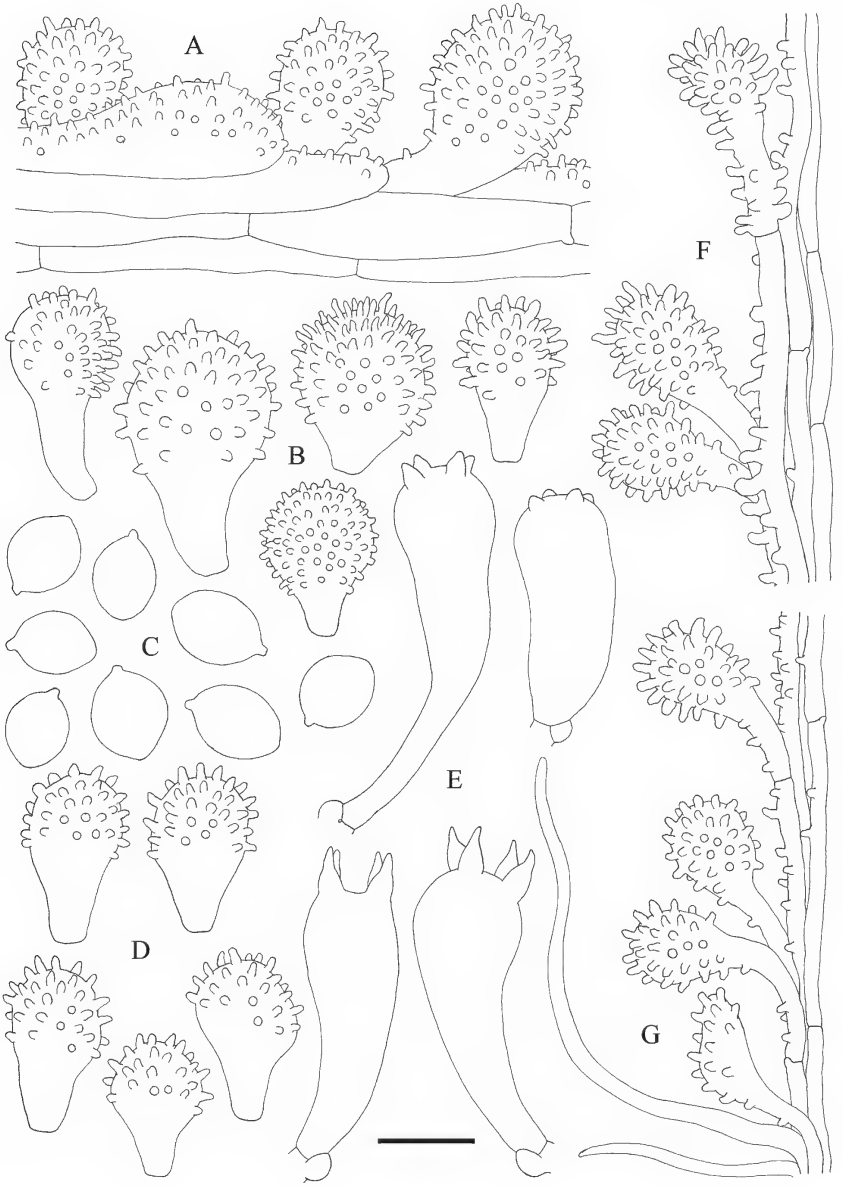

Plate 2— *Mycena acanthophila* microscopic features. A, hyphae of the pileipellis and protruding acanthocysts. B, morphological variation of some detached acanthocysts. C, basidiospores. D, cheilocystidia. E, basidia, including one with a root-like base. F, caulocystidia at the middle part of the stipe. G, caulocystidia at the stipe base, with inconstant smooth hairs. Scale bar = 10 µm.

X-2010, J. Hernanz (AH 40694); Yésero, puerto de Cotefablo, 42°36′57″N 0°12′34″W, alt. 1420 m, on *E. horridum* branches, 12-XII-2010, J. Hernanz (AH 40695). **Castilla la Mancha**: Guadalajara, embalse de Alcorlo, 41°00′36.5″N 3°01′19.8″W, alt. 940 m, on *Erinacea anthyllis* branches, 28-XI-2010, J.C. Campos & J. Hernanz (AH 40696); Somolinos, Sierra de la Pela, 41°15′20.3″N 3°01′19.8″W, alt. 1300 m, on *E. anthyllis* branches, 8-I-2011, J. Hernanz (AH 40697). **Comunidad Valenciana**: Castellón, El Toro, Sierra de el Toro, 39°54′44.87″N 0°48′39.50″W, alt. 1464 m, on *E. anthyllis* branches, 15-X-2010, G. Salvà & S. Català (VAL_MYCO 940). **Navarra**: Lumbier, subida Leyre-Arangoiti, 42°38′40.9"N 1°11′48.9″W, alt. 1340 m, on *E. anthyllis* branches, 31-XII-2010, J. Hernanz (AH 40698).

Comments— *Mycena acanthophila* is placed in sect. *Sacchariferae*, stirps *Alphitophora* based on the pileipellis with acanthocysts, absence of cherocytes, presence of caulocystidia that are densely covered by small warts or spinules, and a stipe without a basal disc (Desjardin 1995). It is well characterised macroscopically by its tiny size, yellowish colouration, comparatively short stipe, and reduced (sometimes absent) lamellae; the often subamygdaliform to citriform basidiospores are also quite unusual.

Two species in sect. *Sacchariferae* with a reduced hymenophore, *M. echinocephala* (G.F. Atk.) Desjardin and *M. cylindrospora* A.H. Sm., also belong to stirps *Alphitophora*. *Mycena echinocephala,* known only from the holotype, differs by the smooth caulocystidia terminated by a spinulose cell [but Desjardin (1993) observed only an amorphous apex when studying the type], unclamped hyphae, different basidiospores, and white basidiomata. *Mycena cylindrospora* can be distinguished by unclamped hyphae, absence of hymenial cystidia, different basidiospores, smooth hymenophore, infundibuliform pilei at maturity, and white basidiomata. Both species grow on *Rhododendron* leaves.

Very few taxa in this section have been described with a yellowish colouration. Perhaps the most similar, *Mycena chloroxantha* Singer, is easily distinguished by the presence of cherocytes in the pileipellis (which places it in stirps *Amparoina*), well-developed close lamellae, ellipsoid basidiospores, very long caulocystidia, and absence of clamp connections. What is more, var. *chloroxantha* exhibits a well-developed, costate basal disc, which is absent in var. *appalachienensis* Desjardin (Desjardin 1995).

More recently described species in stirps *Alphitophora* — *M. dissimilis*, *M. pistacea*, *M. hylophila*, and *M. umbratilis* (Maas Geesteranus & de Meijer 1997, 1998) — all differ in well-developed lamellae, non-yellow basidiomata, and different anatomical features, particularly basidiospores. In addition, *M. dissimilis* and *M. pistacea* lack clamp connections.

Finally, the only two previously known European species of stirps *Alphitophora*, *M. alphitophora* and *M. corynephora*, are readily distinguished by larger white to greyish white basidiomata, well-developed lamellae, basidiospore shape and size (mostly pip-shaped to ellipsoid in *M. alphitophora* and subglobose in *M. corynephora*), and much longer caulocystidia that confer

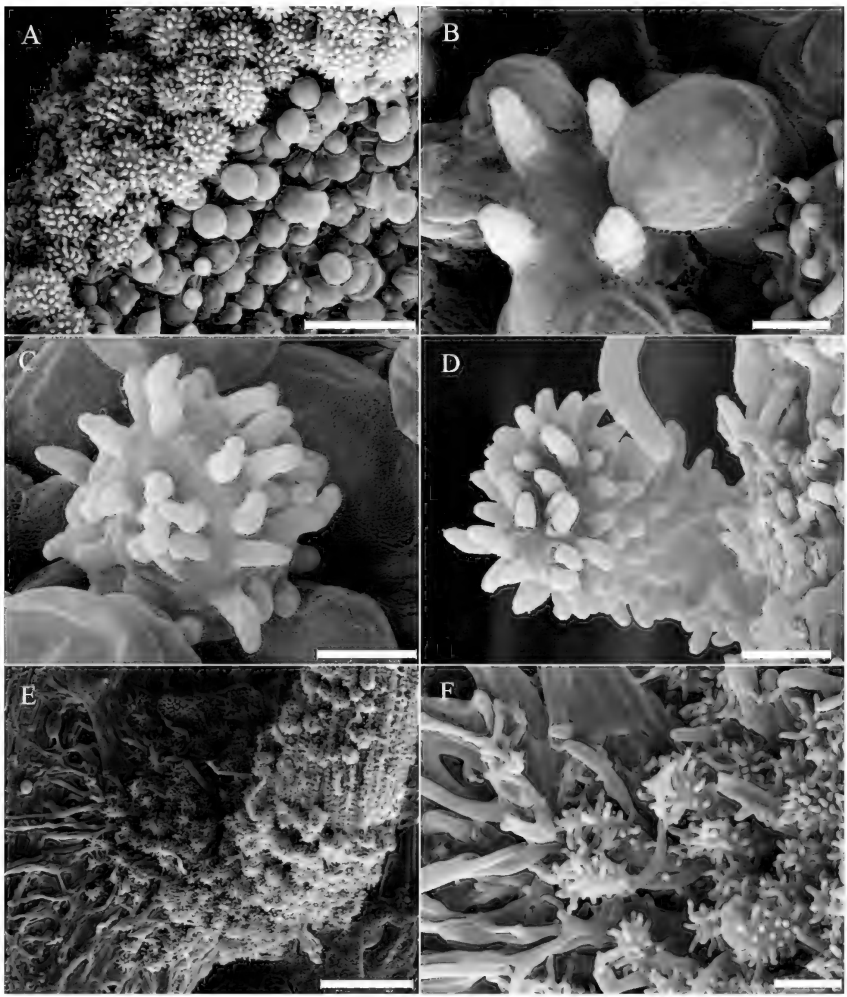

Plate 3— *Mycena acanthophila* SEM photographs. A: pileus margin showing the pileipellis with acanthocysts. B, 4-sterigmate basidia. C, cheilocystidium. D, caulocystidium. E, attachment of stipe with the substratum. F, detail of the stipe base. Scale bars: A = 20 µm; B, C = 3 µm; D = 4 µm; E = 50 µm; F = 10 µm.

a puberulous to hirsute appearance to the stipe surface (Desjardin 1995, Robich 2003).

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Myxomycete history and taxonomy: highlights from the past, present, and future

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Abstract—The past myxomycete legacy covers fruiting bodies preserved in fossil amber more than 35 million years ago and early contributions by Linnaeus, de Bary, and the Listers at the British Museum of Natural History (BM), among whom the Listers introduced monographs, striking watercolors, and more narrow species concepts. Macbride and Martin (University of Iowa) published illustrated myxomycete monographs with broader species concepts. The transfer of the ~9500 specimen Iowa myxomycete collection to the United States National Fungus Collections (BPI) is outlined and ~234 type specimens are listed. Also covered is Lado's recent Nomenmyx and the online resource nomen.eumycetozoa.com, which offers ~900 names for accepted myxomycete species. The current status of the BM and BPI myxomycete collections is noted, and *Didymium saturnus* provides an example of data associated with myxomycete specimens in the American Type Culture Collection. Problematic taxa are recommended for DNA sequencing to help clarify phylogenetic relationships.

Key words—fossils, herbarium, Marie L. Farr, plasmodial slime molds, systematics

The legacy of the past

Myxomycetes preserved in amber— Myxomycetes have a fossil past that dates back to stalked sporangia of *Stemonitis splendens* Rostaf. that are well preserved in Baltic amber from the Tertiary Period and Eocene Epoch approximately 35 to 40 million years ago (Domke 1952; Keller & Everhart 2008). Fossilized stalked sporangia of *Arcyria sulcata* Dörfelt & A.R. Schmidt were also found in Baltic amber from the Eocene (Dörfelt et al. 2003; Dörfelt & Schmidt 2006; Keller & Everhart 2008). The general habit of these myxomycetes are so similar to present-day species that it is apparent that morphology has changed little in 40 million years. Given the advanced fruiting body development observed in these fossils, we can assume myxomycetes have been around much longer than 40 million years.

One can imagine the dominant coniferous forests of *Pinus succinifera* (Göpp.) Conw. that thrived in a warmer subtropical climate and profusely produced resin that accumulated in cracks inside the tree, under the bark, or in wounds around the tree base. This resin eventually flowed and dripped on decaying vegetation, trapping myxomycete fruiting bodies along with a variety of arthropods and plants. The accumulation of Baltic resin (succinite) resulted from millions of years of deposition. As rivers flowed through these forests, this resinous material was carried to form today's deltaic deposits along the Baltic seacoasts at present-day Estonia, Latvia, and Lithuania. However, the Baltic amber region also includes adjacent areas in Denmark, England, the Frisian Islands, Germany, Norway, Poland, Russia (in an area called Samland, in the Kaliningrad Oblast), and Sweden.

Fossil records of fragile myxomycete structures are rare due to the transitory life cycle stages that appear briefly on surface substrata and weather away in a relatively short time. Nevertheless, the excellent state of preservation in myxomycete sporangia representing the *Stemonitales* and *Trichiales* suggests that their geological past might extend to the Paleocene some 65 million years ago when a warmer tropical climate still prevailed.

The 90–94 million year-old New Jersey Cretaceous amber deposits contain the mushroom pileus and stalk of *Archaeomarasmius leggetti* Hibbett et al. that resembles present-day *Marasmius* and *Marasmiellus* mushrooms that are marcescent, persisting and reviving under moist conditions, instead of putrescent, like many fleshy macrofungi (Hibbett et al. 1995; Hibbett et al. 1997; Hibbett et al. 2003). This geological time period has the same tree species and climatic conditions that should also support myxomycete fossils. Discovery of the more ephemeral myxomycete sporangia is remote, and calcareous members of the *Physarales*, the largest and presumably the most morphologically advanced myxomycetes, never have been recorded in the fossil record. However, wherever they are found, amber deposits should be screened by a competent taxonomist for myxomycete fossils to avoid misidentification of confusing and similar looking calicioid lichens (Keller & Everhart 2008). The geological and historical recorded past should be included as part of any review about the myxomycetes.

Early students of the Myxomycetes— The first literature on myxomycetes dates back to 1654 with Pankow's (sometimes spelled Panckow) figure and illustration of the species now known as *Lycogala epidendrum* (L.) Fr. Although Linnaeus' Species Plantarum, published in 1753, is the nomenclatural starting point for the *Myxomycetes*, his treatment of them was best summed up by Martin & Alexopoulos (1969: 18): "Linnaeus, to be sure, knew little about the fungi or slime molds, and cared less." Carl Linnaeus (1701–78), who considered

the myxomycetes to be gasteromycetous fungi (puffballs), was undoubtedly influenced by the appearance of *Lycogala* as a miniature puffball. Martin (1966) provides a more detailed account and review of these early students of the myxomycetes.

The 1800s were marked by a better understanding of the myxomycete life cycle by Heinrich Anton de Bary (1831–88) and the taxonomic advances by his student Józef Thomasz Rostafiński (1850–1928). Earlier myxomycete taxonomists had relied primarily on the striking variation of macroscopic characteristics, whereas Rostafiński (1874, 1875, 1876) ushered in use of microscopic characters that would largely influence later description and classification of the *Myxomycetes*. De Bary has been called the founding father of plant pathology and mycology, which also embraces myxomycology. He was the first to demonstrate myxomycete spore germination and the subsequent stages of myxamoebae, swarm cells, plasmodia, and fruitbody development. De Bary's observations provided the microscopic evidence that led to removal of the *Myxomycetes* from the fungal *Gasteromycetes* and the coinage of the name *Mycetozoa* (Martin 1958; Everhart & Keller 2008).

Arthur and Gulielma Lister, Myxomycetes, and the British Museum (Natural History, now the Natural History Museum)— The lives of the Listers (father Arthur, 1830–1908 and daughter Gulielma, 1860–1949) spanned 119 years. In the main, their world monographs [1894, 1911 (second edition), 1925 (third edition)] follow Rostafiński's classification. The Lister publications stand as a landmark in myxomycete taxonomy, influencing many regional treatises that appeared over the following 50 years. More attention is given to the range of environmental conditions and the remarkable extent to which these factors may influence the appearance and structures of the developing fruiting body. Even so, they recognized 274 species, 101 varieties, and one form for a total of 376 taxa. The more than 60-year influence of the Listers as world authorities on myxomycete taxonomy, especially throughout Europe, resulted in a more frequent use of variety to designate differences in habit, color, and structural microscopic changes in fruiting body morphology. The 128 color and 94 black-and-white plates by Gulielma Lister in the 1925 edition are without equal and stand as a lasting tribute to her artistic skill. Because many species in 53 genera are illustrated in color, they can be easily 'picture-keyed' and correctly identified. The Listers' valuable type myxomycete specimens are deposited in the collections at The Natural History Museum (BM) formerly the British Museum (Natural History), London, United Kingdom.

Macbride, Martin, Myxomycetes, and The University of Iowa— The 1900s highlighted the active research of Thomas Huston Macbride (1848–1934) and George Willard Martin (1886–1971), who served on the faculty at the

University of Iowa (U.S.A.). Their lifetime professional careers spanned almost 100 years (1878–1971) in the Department of Botany at the university. Their field collections, laboratory research, and publications were based largely on specimens housed in the University of Iowa Myxomycete Collection that also included collections of Bethel, Bilgram, Ellis, Farlow, Gilbert, Hagelstein, Harvey, Morgan, Plunkett, Rex, Shimek, and Wingate from America and of Brandza, Japp, Meylan, and Sydow from Europe. Collections were also contributed by O.F. Cook (Liberia) and the Listers (who corresponded and exchanged specimens with Macbride).

The world monograph, "The *Myxomycetes*" by Macbride and Martin was published in 1934 shortly after Macbride's death. The preface by Macbride reads:

> To assist in meeting such inquiry and interest is the purpose of this book. For this edition it has been to the writer's great pleasure and advantage to have the assistance of his colleague, Dr. G. W. Martin: the introduction and general editing of the text is his. By enlarging our field of inquiry, the number of species presented has been greatly enlarged. New plates were needed, – all our plates are new! To the young botanists of our continent and to our contributors around the world, the present volume is especially devoted. May they fare well! T.H.M.

The monograph includes 21 plates and 568 figures, all in black and white. Gladys Baker prepared the habit sketches and a number of her diagrams were made from paraffin sections. Spores, capillitia, and other microscopic structures were drawn by Martin in pencil aided by a camera lucida and using an apochromatic oil immersion lens at a uniform magnification of 1200× to facilitate comparison of these critical characters.

Comparison of the final monographs by Macbride & Martin (1934) with the Listers (Lister 1925) show a trend away from subspecific taxa. Indeed, the Martin introduction emphasizes this trend:

> Decision as to the limits of species and genera must necessarily be a matter of personal judgment, and this is perhaps more apparent in the slime molds, because of their obvious responsiveness to environmental conditions while the fructifications are forming, than in most other groups. We have felt justified in applying a distinctive specific name to any form which shows reasonable constancy under varying conditions, even though occasional intermediate forms may appear. The multiplication of varieties has nearly if not quite reached the saturation point. Many of the varieties listed in the literature are obviously merely growth forms; some are doubtless autonomous species. If the latter, they will eventually be recognized as such; if the former, it would seem better to modify the diagnosis of the species to accommodate them, rather than to multiply names.

The 1934 book recognized a total of 59 genera, 366 species, and 40 varieties. Subjective interpretation was used in some cases to tabulate a variety designation

Fig. 1. Thomas Huston Macbride (1848–1934)
(Courtesy The Scientific Monthly)

because it is not clear whether Macbride merely mentioned the variety name in passing or actually recognized the variety as distinct. In any case the number of varieties is greatly reduced from previous myxomycete authors.

Macbride (Fig. 1), who was heralded as a passionate conservationist and served as the first president of the Iowa Park and Forestry Association, also established the Iowa Lakeside Laboratory at Lake Okoboji in 1909 (Martin 1935). Among the many other honors too numerous to mention are the facts that Macbride served as the tenth president of The University of Iowa (1914–1916) and that the building housing the University of Iowa Museum of Natural History now bears the name of Macbride Hall, dedicated in his honor shortly after his death in 1934 (Martin 1935).

Progress in the present

Martin, Alexopoulos, and The University of Iowa Myxomycete Collection— The tradition of myxomycology at the University of Iowa has involved three outstanding myxomycologists, Macbride, Martin, and Alexopoulos. The world monograph "The *Myxomycetes*" by Martin & Alexopoulos (1969) was another milestone in myxomycete nomenclature and taxonomy. These two myxomycologists worked as a team when Martin was

in retirement and Constantine John Alexopoulos (1907–86) was Chairman of the Department of Botany at the University of Iowa and later served on the faculty at the University of Texas. This book is based on the Macbride–Martin Collection at the University of Iowa that also includes additional numerous collections of C. J. Alexopoulos, Travis E. Brooks, William Bridge Cook, and Donald T. Kowalski.

Perusal and tabulation of all species descriptions yields 53 genera, 425 species, and 2 varieties. The trend here again is away from sub-specific designations, as emphasized by the following:

> One fact should be stressed. The plasmodium may develop its characteristic fruiting stage in less than 24 hours. If this occurs under conditions which cause unduly rapid drying or if repeated rains check the process, great variation may be induced. Under such influences, species which ordinarily have stalks may be sessile or nearly so, or the stalks may be inordinately long; sporangiate species may form plasmodiocarps; aethalioid forms may approach the sporangiate type; the characteristics and disposition of limy secretions may be altered; spore maturation may be checked, resulting in spore-like bodies which are much larger than fully matured spores. Cold weather, and particularly frosts, may induce similar alterations. Such variations are in large part responsible for the extensive synonymy found in the group. Great caution is indicated in describing as new specimens that are the result of such environmental responses. They are not "abnormal"; they are natural responses of the organisms involved to particular stimuli and must be so regarded. Giving them taxonomic status as named varieties serves only to complicate the nomenclature and to extend the meaning of the category variety beyond its legitimate significance. (Martin & Alexopoulos 1969: 3)

This rather long quotation captures the essence of Martin's lifelong approach to the taxonomy of the *Myxomycetes*. This trend in America toward taxonomic conservatism, broader species concepts, and recognition of fewer species was categorized as "species lumping" and those in Europe who had narrow species concepts and described many new species, often based on single collections and limited specimens, were categorized as "species splitters" (Bisby 1953).

All 41 plates and 367 figures show individual images of habits and microscopic characters of crystals, capillitium, and spores that represent a composite set of morphological features. Spores, magnified at 1000×, lack the detail of today's scanning electron microscopic images. Spore ornamentation and other spore characters used in keys relied on high dry power (430×), not the oil immersion lens. This is not apparent in the book text but is what I observed when I was a graduate student during the monograph's preparation. All color illustrations were made by Ruth McVaugh Allen using colored pencils.

The moist chamber culture technique that was introduced at the University of Iowa Mycology Laboratory by Gilbert & Martin (1933) has led to the discovery

Fig. 2. George Willard Martin (1886 –1971)
(Frederick W. Kent, Iowa City, photographer)

of many new tiny myxomycetes found on the bark of living trees. *Echinostelium* de Bary, represented by only a single species, *E. minutum* de Bary, in 1874, increased to five species in 1969 (Martin & Alexopoulos 1969). *Macbrideola*, a genus named after T.H. Macbride by Gilbert (1934), described two new tiny species, *M. scintillans* H.C. Gilbert and *M. decapillata* H.C. Gilbert, obtained from the bark of living trees through moist chamber culture. Thirty-five years later *Macbrideola* was represented by five species (Martin & Alexopoulos 1969).

The chronicle of Martin's life by Lentz & Benjamin (1971) and Wells & Lentz (1973) was augmented by a personal account by Keller (1996). As a United States Army Lieutenant during World War I, Martin was slightly wounded by artillery fire while gathering intelligence on the battlefield during shelling and assaults by the Americans and Germans. During World War II he served as Chief of the Biological Laboratory at the Army Quartermaster Depot in Jeffersonville, Indiana. The Botanical Society of America recognized his extraordinary research and service and he was part of the first group to receive the Certificate of Merit. He served as Director of the Lakeside Laboratory in Iowa from 1928 to 1934. A charter member of the Mycological Society of America, Martin became its first Vice President in 1933 and President in 1944 and also served as Editor-in-Chief of Mycologia from 1950 to 1957. His experience teaching English courses at Rutgers University and his command of narrative text undoubtedly contributed to his 37-year tenure as the editor of the University of Iowa Studies in Natural History from 1934 until his death. The picture of Professor Martin (Fig. 2) was taken about the time of his retirement as head of the Department of Botany in 1955.

He was selected by the North American Flora project as author of The Myxomycetes published by the New York Botanical Garden (Martin 1949). The subsequent world monograph, (Martin & Alexopoulos 1969) is still considered the most authoritative work on the subject. This book along with his lifelong research on myxomycetes were cited as the basis for the Henry Allan Gleason Award given in 1970 to Professor Martin by the New York Botanical Garden for an outstanding recent publication in the fields of plant taxonomy, plant ecology, or plant geography.

During the course of his career he published more than 140 separate titles that included journal papers and books and spanning different groups from dinoflagellates to fungi. The broad spectrum of fungi treated included pathogenic imperfects, water molds, ascomycetes, the *Agaricales*, the resupinates of the *Homobasidiomycetidae*, bird's nest fungi, soil fungi, and tropical fungi from his trips to Colombia, Galapagos Islands, and Panama. Many collections were made of jelly fungi (*Tremellales*) and true slime molds (*Myxomycetes*) while serving as a world authority for these two groups. Most of his papers

were single-authored and his practice of not attaching his name as a coauthor to student-authored papers was followed until his death. Professor Martin was a research mentor for approximately 47 doctoral students (Wells & Lentz 1973), who represented a living testament to his impact on fungi, myxomycetes, and all who knew him professionally.

At the time of Professor Martin's death in 1971, the Macbride–Martin Myxomycete Collection at the University of Iowa numbered approximately 9500 specimens. In a letter dated March 22, 1977 I was informed by Dr. Robert L. Hulbary, Professor and Chairman of the Department of Botany, that the decision not to fill the faculty position held by Professor Martin had resulted in the transfer of the Macbride–Martin Collection of Myxomycetes, January, 1977 to the United States National Fungus Collections (BPI) located at Beltsville, Maryland.

The summer of 1972, I was asked to curate the entire myxomycete collection and compile a list of type specimens. Type specimens were kept in a separate herbarium case that included specimens in boxes as well as microscope slides carefully wrapped in tissue paper inside boxes and all were labeled as types. A list of approximately 235 types (specimens and microscope slides) was prepared from the names taken from the box labels and arranged alphabetically by genus and species. In the following list, authors' names have been corrected and presented as standard abbreviations, where necessary. The following types are now held in BPI:

Amaurochaete *comata* G. Lister & M. Brândză, *A. ferruginea* T. Macbr. & G.W. Martin, *A. minor* Sacc. & Ellis, *A. trechispora* T. Macbr. & G.W. Martin; ***Arcyria*** *annulifera* Torrend (slide only), *A. corymbosa* M.L. Farr & G.W. Martin, *A. magna* Rex; ***Badhamia*** *armillata* Nann.-Bremek., *B. cinerascens* G.W. Martin, *B. dearnessii* Hagelst., *B. gracilis* (T. Macbr.) T. Macbr., *B. iowensis* T. Macbr., *B. viridescens* Meyl.; ***Calonema*** *aureum* Morgan, *C. luteolum* Kowalski; ***Ceratiomyxa*** *morchella* A.L. Welden; ***Clastoderma*** *pachypus* Nann.-Bremek. (slide only); ***Comatricha*** *acanthodes* Alexop. (slide only), *C. aggregata* M.L. Farr, *C. amoena* Nann.-Bremek., *C. brachypus* (Meyl.) Meyl., *C. caespitosa* Sturgis, *C. extendens* Hagelst., *C. longipila* Nann.-Bremek., *C. martinii* Alexop. & Beneke, *C. mirabilis* R.K. Benj. & Poitras, *C. nodulifera* Wollman & Alexop., *C. peritricha* Nann.-Bremek., *C. reticulata* H.C. Gilbert, *C. rispaudii* Hagelst., *C. shimekiana* T. Macbr., *C. subcaespitosa* Peck, *C. suksdorfii* Ellis & Everh., *C. synsporos* Alexop.; ***Diachea*** *silvipluvialis* M.L. Farr, *D. thomasii* Rex; ***Diacheopsis*** *depressa* K.S. Thind & T.N. Lakh.; ***Dianema*** *aggregatum* Kowalski, *D. andersonii* Morgan, *D. harveyi* Rex, *D. nivale* (Meyl.) G. Lister; ***Dictydium*** *rutilum* G. Lister; ***Diderma*** *antarcticum* (Speg.) Sturgis (slide only), *D. brooksii* Kowalski, *D. cinereum* Morgan, *D. corrugatum* T.E. Brooks & H.W. Keller, *D. darjeelingense* K.W. Thind & H.S. Sehgal, *D. indicum* K.S. Thind & H.S. Sehgal, *D. mussooriense* K.S. Thind & Manocha, *D. nigrum* Kowalski, *D. platycarpum* Nann.-Bremek., *D. roanense* (Rex) T. Macbr., *D. rugosum* (Rex) T. Macbr., *D. subcaeruleum* Kowalski, *D. subdictyospermum* (Rostaf.) G. Lister, *D. subincarnatum* Kowalski; ***Didymium*** *aurantipes* T.E. Brooks & Kowalski, *D. discoideum* K.S. Thind & H.S. Sehgal, *D. floccosum* G.W. Martin, K.S. Thind & Rehill, *D. fulvum* Sturgis, *D. labyrinthiforme*

G.W. Martin, Lodhi & N.A. Khan, *D. leoninum* Berk. & Broome, *D. nivicola* Meyl., *D. orthonemata* H.W. Keller & T.E. Brooks, *D. ovoideum* Nann.-Bremek., *D. parietale* G.W. Martin & T.E. Brooks, *D. rugulosporum* Kowalski, *D. saturnus* H.W. Keller, *D. synsporon* T.E. Brooks & H.W. Keller, *D. verrucosporum* A.L. Welden; ***Echinostelium*** *cribrarioides* Alexop., *E. elachiston* Alexop.; ***Enerthenema*** *melanospermum* T. Macbr. & G.W. Martin, *E. syncarpon* Sturgis; ***Hemitrichia*** *karstenii* (Rostaf.) Lister, *H. montana* (Morgan) T. Macbr., *H. obrussea* Meyl., *H. paragoga* M.L. Farr; ***Lamproderma*** *biasperosporum* Kowalski, *L. cristatum* Meyl., *L. fusiforme* Kowalski, *L. gulielmae* Meyl., *L. pulchellum* Meyl., *L. robustum* Ellis & Everh., *L. splendens* Meyl., *L. tuberculospora* M.L. Farr, *L. verrucosum* G.W. Martin, K.S. Thind & Sohi; ***Leocarpus*** *fulvus* T. Macbr.; ***Lepidoderma*** *crustaceum* Kowalksi; ***Licea*** *applanata* Kowalski, *L. biforis* Morgan, *L. denudescens* Keller & Brooks (paratype: TEB2502, as *L colloderma* ined.). *L. erecta* K.S. Thind & Dhillon, *L. fimicola* Dearn. & Bisby, *L. pedicellata* (H.C. Gilbert) H.C. Gilbert, *L. perexigua* T.E. Brooks & H.W. Keller, *L. pseudoconica* T.E. Brooks & H.W. Keller, *L. pumila* G.W. Martin & R.M. Allen, *L. punctiformis* G.W. Martin, *L. scyphoides* T.E. Brooks & H.W. Keller, *L. synsporos* Nann.-Bremek. (slide only), *L. tuberculata* G.W. Martin; ***Lycogala*** *exiguum* Morgan; ***Macbrideola*** *decapillata* H.C. Gilbert, *M. scintillans* H.C. Gilbert; ***Metatrichia*** *horrida* Ing; ***Oligonema*** *aeneum* P. Karst., *O. fulvum* Morgan; ***Orthotricha*** *microcephala* Wingate; ***Paradiacheopsis*** *cribrata* Nann.-Bremek. (slide only); ***Perichaena*** *brevifila* T.E. Brooks & H.W. Keller, *P. quadrata* T. Macbr., *P. syncarpon* T.E. Brooks; ***Physarina*** *echinospora* K.S. Thind & Manocha; ***Physarum*** *auripigmentum* G.W. Martin, *P. bethelii* G. Lister, *P. confertum* T. Macbr., *P. dictyosporum* G.W. Martin, *P. galbeum* Wingate, *P. limonium* Nann.-Bremek., *P. maculatum* T. Macbr., *P. mennegae* Nann.-Bremek., *P. metallicum* Berk., *P. mortonii* T. Macbr., *P. nicaraguense* T. Macbr., *P. nudum* T. Macbr., *P. oblatum* T. Macbr., *P. pulcherrimum* Berk. & Ravenel, *P. retisporum* G.W. Martin, K.S. Thind & Rehill, *P. rubronodum* G.W. Martin, *P. serpula* Morgan, *P. spinulosum* K.S. Thind & H.S. Sehgal, *P. tessellatum* G.W. Martin & M.L. Farr, *P. tropicale* T. Macbr., *P. variabile* Rex, *P. variegatum* K.S. Thind & Dhillon; ***Schenella*** *microspora* G.W. Martin, *S. simplex* T. Macbr.; ***Stemonitis*** *carolinensis* T. Macbr., *S. herbatica* Peck, *S. inconspicua* Nann.-Bremek., *S. morganii* Peck, *S. mussooriensis* G.W. Martin, K.S. Thind & Sohi, *S. nigrescens* Rex, *S. smithii* T. Macbr., *S. uvifera* T. Macbr., *S. virginiensis* Rex, *S. webberi* Rex; ***Trichia*** *andersonii* Rex, *T. cascadensis* H.C. Gilbert, *T. crateriformis* G.W. Martin, *T. erecta* Rex, *T. mirabilis* Nann.-Bremek., *T. reniformis* Peck; ***Tubifera*** *papillata* G.W. Martin, K.S. Thind & Sohi; ***Wilczekia*** *evelinae* Meyl.

Marie L. Farr and the United States National Fungus Collections—Farr received her doctorate from the University of Iowa in 1957 under the direction of Professors G.W. Martin and C.J. Alexopoulos. Thereafter, she spent her professional career at BPI (1958–89), during that time accumulating myxomycete collections from South American countries and the Caribbean Islands between the Tropics of Cancer and Capricorn, a geographic area designated as the Neotropics (Farr 1976). This led to the publication of No. 16 of the Flora Neotropica Monographic Series, a volume devoted to the *Myxomycetes*. The approximately 280 myxomycete species recorded in Flora Neotropica 16 also includes myxomycetes from Florida state (U.S.A.). This myxomycete monograph includes not only the standard dichotomous keys but also offers the first available synoptic keys for the identification of myxomycete species.

It is especially appropriate here to recognize Farr's publications, travels, and collections in the Caribbean Islands and Brazil. She collected myxomycetes during 16 months (1954–55) on the Caribbean island of Jamaica, after which she published an artificial identification key to 26 genera of Jamaican slime-moulds and separate keys to 104 species and 4 varieties (Farr 1957). Recife and the Institute of Mycology at the University of Recife in Brazil was the location of the IMUR herbarium where Farr studied myxomycetes and published a list of 21 genera and 67 species during a stay of several months (Farr 1960). She followed this with an illustrated key to the myxomycetes of South America published in English (Farr 1968) that was the principal South American resource for myxomycete identification. She also participated in the Bredin–Archbold–Smithsonian Biological Survey of Dominica in 1966 that resulted in 500 myxomycete specimens collected in the field (63 from moist chamber cultures), representing 96 myxomycete taxa (Farr 1969).

The first field guide to myxomycetes was written by Farr as part of the How to Know Pictured Key Nature Series launched by Harry E. Jaques in 1937 at Iowa Wesleyan University (Farr 1981). This spiral-bound paperback, even though out of print, is still an excellent source for beginners to identify the more common myxomycete species with dichotomous keys.

Farr updated the Martin & Alexopoulos 1969 monograph in 1983 to provide more current references and information in the introductory topical sections, to reclassify some subclasses, families, and genera, and to add keys and discuss 47 genera under a new title, The genera of *Myxomycetes* (Martin et al. 1983). The 41 plates and 367 figures are the same as those published in the previous world monograph (Martin & Alexopoulos 1969).

Many collections from Dr. D.H. Mitchel and S.W. Chapman, mostly from higher elevations in Colorado, were deposited at the Denver Botanic Garden and BPI in cooperation with Farr (Mitchel et al. 1980). A lifetime of contributions by Dr. Farr to myxomycete taxonomy merits special recognition.

Carlos Lado and Nomenmyx— In 2001, Lado published Nomenmyx, which involved the monumental task of searching the literature for names of myxomycete species. Of the 4000 names applied to various myxomycete taxa he uncovered, 900 are current names for accepted species. These names are now in a database that can be updated over time with the addition of new names and which has been developed into the online resource nomen.eumycetoza.com. Another welcome future addition will be information on types assigned to each myxomycete taxon, the herbarium where the type was deposited, and the transcription of the original description, its geographical distribution, its substratum, and synoptic keys.

Lado (2001) recognized 57 genera and approximately 900 species. Comparison of subspecific taxa was not included but the number of genera closely parallels

that of Nannenga-Bremekamp (1991). As NOMENMYX is strictly a compilation of names that did not involve examination of type specimens, some taxa listed may not represent legitimate myxomycete species; thus rigorous type studies will help refine myxomycete taxonomy. Fortunately some generic names more familiar to students of myxomycetes have been conserved in their more recent usage, including *Amaurochaete* conserved against *Lachnobolus*, *Ceratiomyxa* conserved against *Famintzinia*, *Hemitrichia* conserved against *Hyporhamma*, *Reticularia* conserved over *Enteridium*, and *Tubifera* conserved over *Tubulifera*. All taxonomists are urged to peruse all previous myxomycete names to avoid publishing duplicate names for species new to science.

The promise of the future

MYXOMYCETES AND HERBARIA— Preservation of myxomycete collections in the past usually involved exposure to para-dichlorobenzene (PDB) and/or naphthalene (organic chemical compounds present in mothballs) used to protect against insects, mites, and fungal contaminants. The Macbride–Martin Collection at the University of Iowa was treated with mothballs for many years before concerns that PDB was a potential carcinogen. Furthermore, Gray & Alexopoulos (1968: 228), recommend PDB for use "...in the herbarium case or drawer in which the specimens are stored." This was standard practice for many herbaria of the mid-twentieth century. However, combination of age and treatment with organic agents appears to fragment DNA in older type myxomycete specimens, which impedes DNA amplification for taxonomic molecular studies.

Schenella simplex T. Macbr. was considered a myxomycete for more than 100 years until light microscopy, scanning electron microscopy, and DNA analysis revealed it to be a fungal gasteromycete (Estrada-Torres et al. 2005). The holotype specimen (BPI 839197) was included in a morphological comparison with specimens recently collected in Mexico. DNA extraction was successful for one Mexican specimen, and its nuclear profile most closely matched the fungus *Geastrum saccatum* Fr. whose sequence differed greatly from true myxomycete sequences. Attempts to extract and analyze DNA from the holotype specimen (BPI 839197) using the same molecular techniques were unsuccessful, as the DNA had fragmented and deteriorated over time, either due to age or external treatment with PDB or both (Dennis Miller pers. comm.).

Odell & Perkins (1976) observed that PDB adversely affects life cycle stages of *Didymium squamulosum* (Alb. & Schwein.) Fr. by placing PDB crystals on Petri dish lids, inverting the culture dish, and noting that myxamoebae died in 18 hours leaving ghost-like remnants on the agar surface. Similar treatment of plasmodia stopped protoplasmic streaming within 40 seconds and caused death after 20 minutes. Additional experiments showed that spores from sporangia

of *Comatricha nigra* (J.F. Gmel.) J. Schröt., *Trichia varia* (J.F. Gmel.) Pers., and *T. favoginea* (Batsch) Pers. collected on decaying wood showed a consistent reduction in the percentage of germinated spores when exposed to vapor from PDB crystals. Furthermore, after exposure to PDB, spores were abnormally wrinkled and distorted in shape. These experiments also showed that the percentage of spore germination decreased markedly after only three months. Odell & Perkins (1976) concluded that PDB should not be used in herbarium cases where myxomycete fruiting bodies are stored. Thus, more recently, herbarium managers have had discussions about using type specimens for possible molecular analysis. There is special concern that tiny fruiting bodies of many myxomycete species should not be subjected to destructive techniques used for DNA analysis.

More recent policy changes at BPI will require proof of experience and protocols before granting permission for specimens to be destructively sampled (Amy Rossman, pers. comm.). One possible option is to select an epitype from more recently collected specimens similar to the *Schenella* collections from Mexico (Estrada-Torres et al. 2005). Future molecular techniques may also be developed that can overcome the barriers of age caused by fragmented DNA.

If at all possible, myxomycete type specimens should be deposited in national herbaria that can provide long-term curation and make specimens available to the scientific community. The Natural History Museum (BM) still has a standing policy that specimens, including types, are generally not sent on loan; however, applications to request "invasive sampling" if approved can result in the loan of a small fragment of a fruiting body for SEM observation or DNA extraction. There is justifiable concern that myxomycete fruiting bodies are too small, brittle, and subject to possible damage when sent by mail.

The Lister Myxomycete Collection at BM— The majority of the Lister Myxomycete Collection is housed at BM but a substantial number of specimens are also deposited at the Herbarium, Royal Botanic Garden, Kew (K). Plans are in place to make more information on myxomycete specimens at both BM and K more easily accessible to users. The future of the Lister specimens currently involves the British Mycological Society (BMS) because it is the owner (including copyrights) of the notebooks with the originals of the plates for the monograph and many more unpublished sketches and partly colored illustrations. These notebooks are kept at BM but are administered independently by the library team and not by the Botany Department, which owns and curates only the specimens and microscopic slides. Label information and macro-images of the types will become available on the Internet within the next two years (Holger Thüs, pers. comm.). Consultation with appropriate curators is encouraged to coordinate access to the Lister illustrations and specimens.

The United States National Fungus Collections (USNFC)— The USNFC has a herbarium, a library and extensive databases and web resources that serve as a national and international resource. It is managed under the Systematic Mycology and Microbiology Laboratory (SMML), a federal laboratory of the US Department of Agriculture. There is a good overview of the resources with links to each at http://nt.ars-grin.gov/sbmlweb/fungi/index.cfm. The direct link to the database search pages is found at http://nt.ars-grin.gov/fungaldatabases.

The USNFC herbarium, referred to as BPI (BPI = Bureau of Plant Industry), houses more than one million specimens. About 760,000 herbarium specimens have already been entered into the specimen database, including the *Uredinales* (rusts, 186,925), *Agaricales* sensu lato (45,637 specimens; 718 types), *Ustilaginales* (smuts, 31,175), *Polyporales* (polypores, 91,976), *Deuteromycetes* (imperfect fungi, 127,298), *Ascomycetes* (172,723), and C.G. Lloyd collections (57,086, including myxomycetes) and thus covering most fungi important to agriculture.

BPI is constantly growing and the database is updated accordingly. The BPI Myxomycete Collection, now numbering between 50,000 and 60,000, ranks as the largest in the world. These specimens have been bar-coded (with 12,532 records indexed) and are available on-line at http://nt.ars-grin.gov/fungaldatabases/specimens/specimens.cfm.

The Myxomycete Collection of C.J. Alexopoulos at the University of Texas was transferred to BPI and contains specimens from the state of Texas, U.S.A., Costa Rica, and Greece, along with the D.R. Reynolds collections from the southeastern Asian countries of Thailand, Burma, and the Philippines. The entire Brooks Myxomycete Collection was also transferred to BPI. Many other collectors have deposited myxomycete collections at BPI, but in far fewer numbers.

The American Type Culture Collection— The importance of spore-to-spore culture of myxomycetes and the deposit of living cultures with the United States American Type Culture Collection (ATCC) provides future source materials for scientific experimentation, genetic discovery, and species validation. Good myxomycete taxonomic practice should include the spore-to-spore cultivation of proposed new taxa (See also "Importance of Spore-to-Spore Cultivation and Living Cultures – A Biological Standard," Keller 1996).

The mission of ATCC (www.atcc.org), a private nonprofit biological resource center and research organization, is to acquire, authenticate, preserve, develop, standardize, and distribute biological materials and information for the advancement and application of scientific knowledge. The ATCC Myxomycete Collection (excluding <50 protostelid and >400 dictyostelid strains) is represented by 36 species and slightly less than 200 strains, including the following:

Arcyria elaterensis Mulleavy, *Badhamia gracilis* (T. Macbr.) T. Macbr., *Clastoderma debaryanum* A. Blytt, *Comatricha laxa* Rostaf., *Cribraria violacea* Rex, *Diachea leucopodia* (Bull.) Rostaf., *Didymium difforme* (Pers.) Gray, *D. iridis* (Ditmar) Fr., *D. nigripes* (Link) Fr., *D. squamulosum* (Alb. & Schwein.) Fr., *Echinostelium arboreum* H.W. Keller & T.E. Brooks, *E. coelocephalum* T.E. Brooks & H.W. Keller, *E. minutum* de Bary, *Metatrichia vesparium* (Batsch) G.W. Martin & Alexop., *Physarum cinereum* (Batsch) Pers., *P. compressum* Alb. & Schwein., *P. didermoides* (Pers.) Rostaf., *P. flavicomum* Berk., *P. melleum* (Berk. & Broome) Massee, *P. polycephalum* Schwein., *P. pusillum* (Berk. & M.A. Curtis) G. Lister, *P. rigidum* (G. Lister) G. Lister, *P. roseum* Berk. & Broome, *Stemonitis flavogenita* E. Jahn, *Willkommlangea reticulata* (Alb. & Schwein.) Kuntze

Each accessioned strain is assigned an ATCC number with associated information about the live culture and the origin of the collection. *Didymium saturnus* H.W. Keller is presented as an example (Keller 1970):

> ATCC® Number, Order this item, 64178™, Organism, *Didymium saturnus* H.W. Keller, Designations, HWK 132, Isolation, Oat straw, Iowa, Depositors, H.W. Keller, Biosafety Level, 1, Shipped, frozen, Growth Conditions, ATCC medium: Corn meal agar, half-strength, Temperature: 24.0°C, Duration: grown with *Escherichia coli* ATCC 23437, Permit/Forms, In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location, Type Strain, yes, References, 13457: Keller HW. *Didymium saturnus*, a new myxomycete occurring on straw stacks. Mycologia 62: 1061–1066, 1970. Three other collections are also available as live, two-membered cultures deposited by HWK as ATCC 64199 *Badhamia rhytidosperma* H.W. Keller & Schokn. (Keller & Schoknecht 1989c); ATCC 64200 *Didymium annulisporum* H.W. Keller & Schokn. (Keller & Schoknecht 1989a); ATCC 64201 *Badhamia spinispora* (Eliasson & N. Lundq.) H.W. Keller & Schokn. (Keller & Schoknecht 1989b). Hardcopy books are no longer available thus relevant information can be accessed online or by telephone. Deposit of myxomycete live cultures still is possible and relevant information can be found at the ATCC website. Specific criteria can be viewed online but consideration should be given as follows: "The expected demand or need for all potential deposits is reviewed and prioritized with input from the scientific community and is now a key factor in our acquisition protocol."

DNA SEQUENCING OF PROBLEMATIC MYXOMYCETE TAXA— Future directions using DNA sequencing techniques will surely unlock many phylogenetic mysteries among the five myxomycete orders (*Echinosteliales, Liceales, Trichiales, Stemonitales, Physarales*) plus a number of genera difficult to classify. Some of these perplexing myxomycete taxa are monotypic (monospecific) genera with a distinctive suite of characters. Several examples will serve to pinpoint the taxonomic problems and to suggest a priority list of taxa that should receive a higher priority for DNA sequencing.

Minakatella longifila G. Lister is a monotypic genus that has resided in the *Trichiales* since described in 1921 within *Arcyriaceae,* a family with tubular (hollow) capillitial threads; the Listers (1925) considered *Minakatella* close to

Perichaena. Martin and Alexopoulos (1969) included the genus in *Dianemaceae* (*Dianemataceae*), emphasizing the apparent solid character of the capillitial threads. After ultrastructural evidence showed clearly that the capillitial threads of *Minakatella* were hollow, it was therefore transferred to the family *Trichiaceae* (Keller et al. 1973).

Nannenga-Bremekamp (1982) used polarized light and the birefringent property of spores and capillitium that triggered taxonomic changes for *Minakatella*. The lack of birefringence of the capillitial threads led to removal of *Minakatella* from *Trichiales* and establishment of a new family, *Minakatellaceae,* in the *Liceales*. Birefringence of the capillitium using the Nannenga-Bremekamp data is not constant (present and absent) within *Trichiales,* however, as demonstrated by *Arcyria* and *Perichaena* capillitial threads with little or no birefringence and *Trichia* and *Hemitrichia* with the highest measured levels of birefringence, all genera are still retained in the *Trichiales*. Ing (1999: 365) does not follow Nannenga-Bremekamp's taxonomy, although he recognized her taxonomic rank of the *Minakatellaceae* in the *Trichiales*. This is an extreme example of the application of "tyranny of a character" or "taxonomic tinkering" that has led to taxonomic confusion, proliferation of families, and the need for DNA analysis to help solve the final disposition of questionable taxa.

More taxa that should be considered a higher priority for DNA analysis because of uncertain taxonomic position are: *Kelleromyxa fimicola* (Dearn. & Bisby) Eliasson, *Listerella paradoxa* E. Jahn, *Protophysarum phloiogenum* M. Blackw. & Alexop., and *Trabrooksia applanata* H.W. Keller. These taxa are rare and seldom collected, for example, the occurrence of fruiting bodies of *K. fimicola* on herbivorous dung, *L. paradoxa* on *Cladonia* lichens, and *T. applanata* on the bark of living trees (especially *Juniperus virginiana* L.). This requires due diligence to find specimens in specific habitats capable of yielding positive results for DNA analysis. More taxa that would help clarify generic positions include *Barbeyella minutissima* Meyl., *Calomyxa metallica* (Berk.) Nieuwl., *Cornuvia serpula* (Wigand) Rostaf., *Badhamiopsis ainoae* (Yamash.) T.E. Brooks & H.W. Keller, *Erionema aureum* Penz., *Leocarpus fragilis* (Dicks.) Rostaf., and *Physarella oblonga* (Berk. & M.A. Curtis) Morgan. These and other taxa would provide valuable information for species currently granted an uncertain taxonomic status. The future of myxomycete biosystematics will depend more and more on DNA analysis, and I hope this will come sooner rather than later.

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Synonymy of *Suillus imitatus*, the imitator of two species within the *S. caerulescens/ponderosus* complex

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Abstract—Identification of three species of *Suillus*, *S. caerulescens*, *S. ponderosus*, and *S. imitatus*, has always been difficult because of overlapping and non-discrete morphological characters. To solidify the identification of these taxa, we compared the nucleotide sequences from the internal transcribed spacer region (ITS) of the type specimens of *S. caerulescens*, *S. ponderosus*, *S. imitatus* var. *imitatus*, and *S. imitatus* var. *viridescens* with field collected specimens which we identified as *S. caerulescens, S. ponderosus,* and *S. imitatus* in northern California. Based on ITS sequence identity and phylogenetic inference, specimens of *S. caerulescens* and *S. ponderosus* formed well-supported clades with the holotype of the respective species. However, *S. imitatus* var. *imitatus* fits within the *S. caerulescens* clade and *S. imitatus* var. *viridescens* fits within the *S. ponderosus* clade. Therefore, we synonymize *S. imitatus* var. *imitatus* with *S. caerulescens* and *S. imitatus* var. *viridescens* with *S. ponderosus,* and show that the species can indeed be recognized morphologically based on annulus characteristics.

Key words—bolete, phylogenetics

Introduction

Suillus Gray is a large ectomycorrhizal genus in the *Boletales* with about 170 known species (Index Fungorum and Mycobank); about half of those species are found in North America (Bessette et al. 2000) and about 40 species in the western United States (Smith 1979). The four taxa in this study, *S. caerulescens*, *S. ponderosus*, *S. imitatus* var. *imitatus,* and *S. imitatus* var. *viridescens* (all within section *Boletinus*), are restricted to the North American west coast. Smith & Thiers (1964) described *S. caerulescens, S. ponderosus,* and *S. imitatus* as three distinct species based mainly on texture of the annulus/annulus remnant and pileus color. Later, Smith & Trappe (1972) described *S. imitatus* var. *viridescens* from western Oregon based on the strong greening reactions of cut tissue in the

Table 1. Morphological comparisons of four *Suillus* taxa. *

	S. caerulescens	*S. ponderosus*	*S. imitatus* var. *imitatus*	*S. imitatus* var. *viridescens*
Holotype	MICH12297 (Smith 48733)	MICH12305 (Smith 20204)	MICH12300 (Smith 48732)	MICH12301 (Smith 78754)
Pileus size	6–14 cm	9–25 cm	4–12 cm	4–12(–15) cm
Pileus color	Vinaceous, ochraceous tawny to ochraceous buff	Deep vinaceous brown to testaceous	Orange cinnamon to dingy cinnamon	Reddish orange, ferruginous, dull to cinnamon rufous to cream-buff
Tube mouths (mm)	1–2	1–3	2–3	~ 1
Stipe size (cm)	2.5–8 × 2–3	9–14 × 3–6	3.5–6 × 1.5–2.5	3–9 × 1–3
Stipe staining	Base slowly blue when cut	Surface blue to greenish	Base surface (1/2–1/3) quickly blue when cut	Surface blue and/or green in moist (not dry) specimen
Annulus	Band-like, fibrillose, pallid to white, not gelatinous	Membranous, reddish-cinnamon, gelatinous	Band-like, dingy pallid, staining brownish, felty-tomentose (edges with some gluten)	Wide band with buff-orange slimy edge, fragmentary in age
Spores (as reported)	8–11 × 3–5 µm	8–10(–12) × 3.8–5 µm	7–9 × 4–4.5 µm	8–11 × 4–4.5 µm
Spores (as measured)	7.6–10.2 × 4–4.4 µm	6–8.4 × 4–4.2 µm	7–10 × 4–4.5 µm	7–11 × 4–4.4 µm

* Adapted from Smith (1979), Smith & Thiers (1964), and Smith & Trappe 1972. Spore dimensions are reported based on the literature and as measured in this study.

stipe and the strong green colored specimens in the field. Table 1 provides an overview of the morphological characteristics of these four taxa.

The four taxa are recognized in monographs and western guidebooks (e.g. Arora 1986, Bessette et al. 2000, Smith & Thiers 1964, Thiers 1967, 1975). *Suillus caerulescens* and *S. ponderosus* are very common mycorrhizal associates of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), while *S. imitatus* was described as a spruce associate (Smith & Thiers 1964) and is listed in modern field guides as occurring in mixed conifer forests in the Pacific Northwest (Bessette et al. 2000). In many years, fruit bodies belonging to the *caerulescens/ponderosus* complex can be found in great abundance in coastal California forests. As a result, it is not unusual to come across these taxa and to be faced with the challenge of identifying them correctly. In the field, we rely heavily on annulus characters to differentiate between species (Fig. 1), but this is a short-lived, developmental character that can be altered or at least obscured by age

FIGURE 1. *Suillus caerulescens* (A) and *S. ponderosus* (B): Comparison of veil and other morphological characters.

and weather. Prof. Harry Thiers used to joke that in dry weather all specimens had a dry annulus and were called *S. caerulescens* and in wet weather the annuli were more gelatinous and the species was called *S. ponderosus*.

In contrast, the name *S. imitatus* is rarely applied to California material, except to those collections that have a greenish tint and are referred to as *S. imitatus* var. *viridescens* (Arora 1986). The original description of *S. imitatus* var. *imitatus* compared it primarily with *S. lakei* (Murrill) A.H. Sm. & Thiers, but field guides usually compare it with *S. caerulescens* from which it is distinguished with difficulty, by its supposedly slightly shorter spores and slightly more orangish pileus (Thiers 1975, Arora 1986, Bessette et al. 2000). *Suillus lakei,* another Douglas-fir associate closely related to the *caerulescens/ ponderosus* complex (Kretzer et al. 1996), is easily distinguished by its reddish, fibrillose pileus. To help sort out the species present in our area, we combined existing GenBank ITS sequences with newly acquired ITS sequences from the holotypes and multiple collections of *caerulescens/ponderosus* complex.

Materials & methods

Our field collections were obtained from northern California (TABLE 2). We obtained distribution information from our field-collected specimens, those deposited in the University and Jepson Herbarium at the University of California, Berkeley (UC), photos deposited in the online database Mushroom Observer that have clear identification, and available checklists for Arizona (Bates 2006), Montana (C. Cripps pers. comm.), and the Intermountain Region (Piep 2003). These sources combined to provided an acceptable account for the overall natural distribution of the species studied here.

We extracted DNA from freshly collected specimens (UC 1861375-UC 1861389) using a modified Sigma Extract-N-Amp kit (Sigma Aldrich, St. Louis, MO). For

Table 2. Specimens sequenced in this study.

Species	Collection date	Collection location*	Herbarium^ #	GenBank #
S. caerulescens	3 Dec. 2006	CA: San Francisco Bay area	UC 1861383	JQ958308
	3 Dec. 2006	CA: San Francisco Bay area	UC 1861384	JQ958309
	3 Dec. 2006	CA: San Francisco Bay area	UC 1861385	JQ958310
	3 Dec. 2006	CA: Marin Co., Mill Valley, Mt. Tamalpais	UC 1861386	JQ958311
	3 Dec. 2006	CA: Marin Co., Mill Valley, Mt. Tamalpais	UC 1861387	JQ958312
	3 Dec. 2006	CA: Marin Co., Mill Valley, Mt. Tamalpais	UC 1861388	JQ958313
	3 Dec. 2006	CA: Marin Co., Mill Valley, Mt. Tamalpais	UC 1861389	JQ958314
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861378	JQ958315
S. ponderosus	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861375	JQ958320
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861376	JQ958321
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861377	JQ958322
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861379	JQ958323
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861380	JQ958324
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861381	JQ958325
	19 Nov. 2006	CA: Mendocino Co., JDSF, Co. Rd 409 & Little Lake Rd., 39.3256 N 123.7372 W	UC 1861382	JQ958326
S. caerulescens, holotype	9 Oct. 1954	WA: Pierce Co, Spanaway Park	MICH 12297	JQ958316
S. imitatus var. *imitatus*, holotype	9 Oct. 1954	WA: Pierce Co., Mt. Rainier National Park, Power House Woods	MICH 12300	JQ958317
S. imitatus var. *viridescens*, holotype	2 Oct. 1970	OR: Lincoln Co., near Otis, Van Duzer Corridor	MICH 12301	JQ958319
S. ponderosus, holotype	24 Oct. 1944	OR: Clackamas Co., Rhododendron	MICH 12305	JQ958318

*Location abbreviations: JDSF = Jackson Demonstration State Forest.

^Herbarium codes: UC = University and Jepson Herbaria, University of California, Berkeley; MICH = University of Michigan Herbarium.

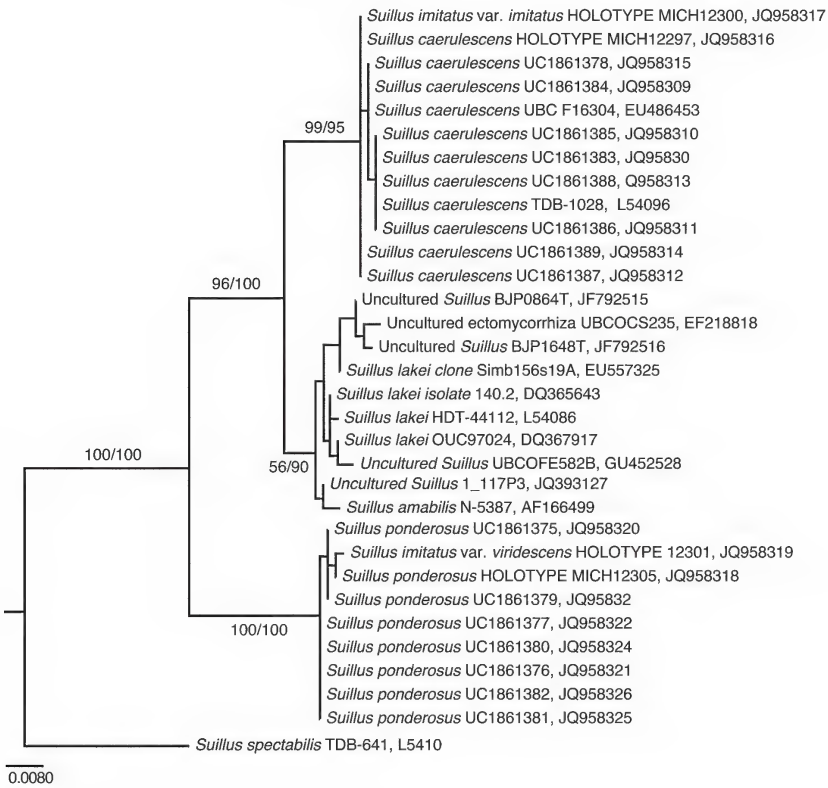

FIGURE. 2. ITS-sequence based maximum likelihood tree of the *Suillus* species in this study. Maximum likelihood followed by maximum parsimony bootstraps provide statistical support for branches with >50% support. Each sequence contains the isolate/specimen number followed by a GenBank accession number. *Suillus spectabilis* served as the outgroup.

specimens that had been stored in a herbarium, it was necessary to obtain high quality DNA so we used a standard CTAB buffer lysis followed by chloroform extraction and ethanol precipitation. DNA was extracted from the following herbarium type specimens: MICH 12305, MICH 12297, MICH 12300 and MICH 12301. Herbarium abbreviations are according to Thiers (2012; continuously updated). For field/recently collected specimens, we used the standard fungal specific primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). For herbarium specimens with degraded DNA we amplified the ITS gene in two segments when necessary. We used standard ITS and *Suillus* ITS specific primers (ITS-2S and ITS-3S) in various combinations: ITS1F and ITS2S or ITS2 and ITS3S or ITS3 and ITS4 (Bruns et al. 2010). Sequences were produced using the standard BigDye Terminator v3.1 Kit and ran on an ABI Prism 3700 Genetic Analyzer (Life Technologies).

We examined each automated sequence and manually interpreted and corrected ambiguous bases using Sequencher (Gene Codes Corporation). These sequences were deposited in GenBank under accession numbers (JQ958308-JQ958326). Using BLAST we gathered from GenBank all sequences similar to the species mentioned as of April 24, 2012: *S. lakei* (GenBank L54086, DQ365643, DQ367917, EF218818, AF166499, GU452528, JF792516, EU557325, JQ393127, JF792515), *S. caerulescens* (GenBank L54096, EU486453), and *S. spectabilis* (Peck) Kuntze (GenBank L54104) was used as an outgroup species. We aligned the sequences manually and analyzed the alignment using the maximum likelihood model GTRGAMMA in RAxML (Stamatakis 2006). Maximum likelihood bootstrap was performed using the RAxML online BlackBox interface (Stamatakis et al. 2008). Maximum parsimony bootstrap was performed using PAUP v.4.0b6 with 1000 replicates (Swofford 2001). Corrected distances between sequences were found using the Kimura 2-parameter in PAUP (Swofford 2001).

Results & discussion

We obtained full length ITS coverage for three of the four type specimens along with the 15 specimens that we collected for this study. We were able to obtain only the ITS1 and ITS2 regions of the *S. caerulescens* holotype MICH12297. We combined the sequences generated with GenBank sequences of *S. lakei*, and the outgroup sequence from *S. spectabilis*. The matrix contained 32 taxa, 613 characters, and 30 parsimony informative characters. The resulting maximum likelihood tree is shown in Fig. 2.

To our surprise specimens that we had determined as *S. caerulescens* and *S. ponderosus* based on annulus differences (Fig. 1) separated into two clades with good statistical support (Fig. 2). Despite the morphological similarities between these two species, *S. caerulescens* is sister not to *S. ponderosus,* but to *S. lakei*. Together these three species form a clade with moderately high support (85%) based on an analysis of 40 *Suillus* taxa (data not shown). From these results, we conclude that annular characters, when present, are a reliable way to separate *S. caerulescens* from *S. ponderosus* (Table 1, Fig. 2).

In contrast, *S. imitatus* sequences did not fall within their own clade but fit within the clades for the two other species: *Suillus imitatus* var. *imitatus* within the *S. caerulescens* clade, and *S. imitatus* var. *viridescens* within the *S. ponderosus* clade. At least so far, morphologically defined *Suillus* species separate fairly well by ITS, but we know from other taxa that not all species may be distinguished by differences within the ITS region (Manian et al. 2001, Bruns et al. 2010). This prompts the question of whether there are morphological reasons to suspect that one or both *S. imitatus* varieties might be distinct at the species level. Interestingly, in the discussion that followed separation of the *S. imitatus* varieties, Smith & Thiers (1964) and Smith & Trappe (1972) initially thought that *S. imitatus* var. *imitatus* might represent *S. caerulescens* and *S. imitatus* var. *viridescens* might be *S. ponderosus*. They decided instead to recognize two

taxa separated by pileus color and spore size differences. The shorter spores for *S. imitatus* var. *imitatus* reported by Smith & Thiers (1964) and cited in many later references were given as 7–9 × 4–4.5 µm. Although this overlaps the lower end of the spore range given for *S. caerulescens* (8–11 × 3–5 µm), it could indicate a subtle distinction. However, we re-measured the spores of the type collection as 7–10 × 4–4.5 µm, which now puts the var. *imitatus* spores within the center of the range for *S. caerulescens, S. ponderosus,* and *S. imitatus* var. *viridescens.* The orangish pileus color of *S. imitatus* var. *imitatus* certainly overlaps the highly plastic pileus colors seen in *S. caerulescens* and *S. ponderosus,* and the greenish tint of *S. imitatus* var. *viridescens* could easily be environmentally induced, much as the aqua colors seen in the stipe of *Leccinum* species (den Bakker & Noordeloos 2005). For these reasons we see no compelling argument to maintain either variety of *S. imitatus* as a separate taxon and so synonymize var. *imitatus* with *S. caerulescens* and var. *virescens* with *S. ponderosus*.

Suillus lithocarpi-sequoiae was described by Singer (1960) from Muir Woods, Marin County, California. The descriptions for this species fit well within our current concept of *S. ponderosus*. The type specimen (Singer N 1531) was deposited in Fundación Miguel Lillo, Argentina (LIL). Unfortunately, the type specimen could not be located and is assumed lost. Singer placed (1962) and maintained (1975, 1986) this species in *Pulveroboletus,* even though Smith & Thiers (1964) and Thiers (1975) thought it better placed in *Suillus*. The species was never encountered again at the type locality, despite repeated search efforts (Thiers 1975). For these reasons we consider the name *S. lithocarpi-sequoiae* doubtful.

Below is an updated taxonomy, and emended diagnoses for *S. caerulescens* and *S. ponderosus*.

Taxonomy

Suillus caerulescens A.H. Sm. & Thiers, Contr. Monogr. N. Amer. *Suillus*: 36, 1964. FIGURE 1A

HOLOTYPE: MICH12297 (A.H. Smith 48733); GENBANK nrITS JQ958316.

= *Suillus imitatus* A.H. Sm. & Thiers var. *imitatus*, Contr. Monogr. N. Amer. *Suillus*: 40, 1964.

HOLOTYPE: MICH 12300 (A.H. Smith 48732); GENBANK nrITS JQ958317

These two names were validly published at the same time and therefore have equal priority. However, we have chosen to retain the name *S. caerulescens* over *S. imitatus* because of its broader use in many research articles, guidebooks, and Internet web pages.

CHARACTERISTICS—Annulus band-like, fibrillose to felty-tomentose, white to dingy pallid, staining brownish, not gelatinous to slightly gelatinous with some gluten along the edges.

Suillus ponderosus A.H. Sm. & Thiers, Contr. Monogr. N. Amer. *Suillus*: 38, 1964. FIGURE 1B

HOLOTYPE: MICH12305 (A.H. Smith 20204); GENBANK nrITS JQ958318

= *Suillus imitatus* var. *viridescens* A.H. Sm. & Trappe, Mycologia 64: 1151, 1972.

HOLOTYPE: MICH12301 (A.H. Smith 78754); GENBANK nrITS JQ958319

CHARACTERISTICS—Annulus membranous and band-like, buff-orange to reddish-cinnamon, gelatinous to highly gelatinous and slimy along the edge.

COMMENTS— Both species seem to occur within the host range of Douglas fir (*P. menziesii*). *Suillus caerulescens* occurs from coastal northern California north to British Columbia (Canada) and Montana, and in Arizona. It has also been found in the northern Sierra Nevada at lower elevations where *P. menziesii* occurs (E.C. Vellinga pers. obs.). There is one report of its occurrence in northern San Diego County, CA, on Palomar Mountain (Mushroom Observer 2012: 61662). However, *Pseudotsuga macrocarpa* (Vasey) Mayr rather than *P. menziesii* occurs there. Unfortunately this material was not available for study. *Suillus ponderosus* occurs in coastal areas from northern California (north of Monterey Bay) to northern Oregon, but it is unclear whether it occurs in the Sierra Nevada. *Suillus lakei,* sister to *S. caerulescens*, occurs in coastal northern California, north into Montana, British Columbia, and Alberta (Canada) and in patchy areas of the Rocky Mountains in Colorado, Utah, New Mexico and Arizona (Mushroom Observer, Piep 2003, Bates 2006). This wide distributional range may explain the ITS variations seen in FIG. 2.

To understand ITS genetic variability within and among the three species in this study, we determined the intraspecific and interspecific percent sequence similarity based on the corrected Kimura 2-parameter. Together with the type specimens, all sequences within each of the three clades show high intraspecific similarity: *S. caerulescens* (99.8–100%), *S. ponderosus* (99.5–100%), *S. lakei* (98.4–100%). However, the interspecific similarity can be as high as 98.8% among species: *S. caerulescens* (93.8–98.8%), *S. ponderosus* (93.8–96.8%), *S. lakei* (94.3–98.8%). This is another case in which strict ITS similarity [the 97% sequence similarity that serves as a proxy for operational taxonomic unit (OTU) separation in ecological studies] does not necessarily correctly differentiate species. Further study is necessary to determine the ITS genetic variability for the whole genus *Suillus*. However, based just on what we already observed here, caution is needed when using the broad implementation of the 97% unilateral separation for all taxa (reflected in Hibbett et al. 2011, and references therein). We strongly recommend that a phylogenetic framework be used for species delimitation in studies using DNA sequences.

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We thank the Herbarium at the University of Michigan for providing loans of type specimens and the two reviewers, Boris Assyov and Dimitar Bojantchev, for their useful comments to improve the manuscript. Support for this research was provided by the NSF-GRFP to NHN and a Sonoma County Mycological Association (SOMA) Graduate Scholarship to JFK.

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Phylogenetic relationships and the newly discovered sexual state of *Talaromyces flavovirens*, comb. nov.

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Abstract — Typical *Talaromyces* ascomata were observed on dry *Quercus suber* leaf litter amongst the characteristic synnemata of *Penicillium aureocephalum*, and they appear to represent the sexual state of the latter species. The species is a synonym of the older *Lasioderma flavovirens*, and we propose the new combination *Talaromyces flavovirens*. Lectotype and epitype specimens are designated for this name. The defining characters of the asexual state include yellow, short-stalked, mycetozoan-like synnemata with an unusual, almost closed terminal head of penicillate conidiophores intermixed with sinuous hyphae, and dark green conidia. Ascomata could not be induced in culture, but PCR amplifications of mating-type genes indicate the species is heterothallic. In nature, ascocarp initials appear to be antheridia coiled around clavate ascogonia, similar to those of *T. flavus*, and the thick-walled, spiny ascospores are also similar to those of *T. flavus*. ITS barcodes and β-tubulin sequences place *T. flavovirens* in a clade with *T. apiculatus*, *T. flavus*, *T. funiculosus*, *T. galapagensis*, *T. pinophilus*, *T. macrosporus*, and seven other species.

Keywords — anamorph-teleomorph connections, DNA barcoding, hyphomycetes, *Trichocomaceae*.

Introduction

Penicillium aureocephalum was described as a new species of synnematous hyphomycete from Catalonia, Spain (Muntañola-Cvetković et al. 2001). It typically occurs on *Quercus suber* and *Cistus salviifolius* leaf litter, producing synnemata that resemble stalked mycetozoan sporangia. Although difficult to observe without micro-dissection, the synnemata have symmetrical

conidiophores typical of *Talaromyces* (formerly referred to as *Penicillium* subg. *Biverticillium*), with long, thin acerose phialides and a phialide:metulae length ratio of about 1:1 (Pitt 1979). In culture on malt extract agar, *P. aureocephalum* typically produces hirsute yellow structures that resemble ascomata of *Talaromyces,* but they do not mature, even after months of incubation (Muntañola-Cvetković et al. 2001).

A subsequent study of the distribution of *P. aureocephalum* in Spain yielded abundant collections on *Quercus* leaves (Llimona et al. 2006). On some specimens, ascocarps resembling those of *Talaromyces,* with prolate spiny ascospores, were observed among the synnemata. Subsequent isolations from these asci resulted in the production of synnemata cultivation of *P. aureocephalum* in the pure cultures. This, the habitat specificity and the sometimes intertwined biverticillate conidiophores and asci led to our conclusion that the ascomata represent the sexual stage of *P. aureocephalum*.

Montagne (1845) described the monotypic genus *Lasioderma,* with its type species *L. flavovirens* collected on *Quercus suber* leaf litter from Algeria. The original description reads, "Peridium e turbinato obpyriforme e floccis septatis ramosis laxe intricatis contextum, subpersistens. Sporæ minutæ, numerosisimæ, contiguæ, olivaceæ in centro evanescente peridii conglutinate." The species diagnosis adds only a few details, "stipite rufo statim in capitulum globosum luteum dilatato," then refers to an 'ic. ined.' The color illustration was published the following year in Flore d'Algérie Durieu (1846). The corresponding Flore d'Algérie text (Durieu 1848) presented an essentially identical diagnosis, additional details of a location near la Calle, Algeria (i.e. on the Mediterranean coast), details of an earlier Spanish collection made in 1824 by Durieu (selected as lectotype below), an earlier unpublished manuscript name ("*Cephalotrichum flavovirens* Durieu," not to be confused with the validly published name *Cephalotrichum flavovirens* (Alb. & Schw.) Nees), and clarification of how *Lasioderma* differed from *Trichoderma.*

The original and expanded descriptions (Montagne 1845, Durieu 1848), color plate (Durieu 1846; reproduced in MycoBank MB120473), specific habitat, and geographical location are all suggestive of *P. aureocephalum*. Its specific habitat and the striking morphological similarities between the Durieu (1846) sketch and *P. aureocephalum* synnemata led us to believe that they might be the same fungus. The suspicion was confirmed by our examination of authentic material in PC, from which we selected the lectotype below.

Seifert et al. (2012) reviewed the historical literature on *Lasioderma*, tracing its fate through Saccardo's (1886) and Lindau's (1900) hyphomycete compilations until it was deflected into the *Basidiomycetes* by Höhnel (1910) and then largely forgotten. We rediscovered the name because of its inclusion in the host index by Oudemans (1920). Because *Lasioderma* is an older name than *Talaromyces*,

and *Lasioderma* is also the current name of a genus of beetles, Seifert et al. (2012) proposed the conservation of *Talaromyces* over *Lasioderma*. However, *L. flavovirens* remains a valid species name, predating *P. aureocephalum* by more than 150 years. The recent move to single name nomenclature has placed *Penicillium* subg. *Biverticillium* in synonymy with *Talaromyces* (Houbraken & Samson 2011). Therefore, in this paper we introduce the new combination *Talaromyces flavovirens* and report our efforts to document the connection of the newly recognized sexual stage and its better-known synnematous stage. To supplement the recent phylogenetic analysis of *Talaromyces* by Samson et al. (2011), we also present the results of ITS and β-tubulin sequencing of multiple strains of this fungus, demonstrating its coherence as a phylogenetic species and confirming its relationships with other asexual *Talaromyces* species.

Materials & methods

Cultures

Quercus suber leaf litter with visible colonies of *T. flavovirens* was collected in Catalonia, Spain by XL and his collaborators. Cultures were isolated from conidial masses from these specimens by KAS. Dried specimens are deposited in the Canadian National Mycological Herbarium (DAOM) and the cultures in the Canadian Collection of Fungal Cultures (CCFC) under accession numbers DAOM 236381–DAOM 236383.

Morphology

The fungus was characterized from its natural habitat (*Q. suber* leaves) and by using standardized cultivation techniques on Czapek Yeast Autolysate agar (CYA), Difco Malt Extract agar (MEA; Pitt 1979, Okuda et al. 2000) and Yeast Extract Sucrose agar (YES, Frisvad & Samson 2004). Spore suspensions were prepared in semi-solid 0.2% agar with 0.05% Tween80 and inoculated onto 9 cm Petri dishes in three-point pattern using a micropipette. All plates were incubated in the dark for 7 days at 25°C, with additional CYA plates incubated at 5 and 37°C. Color names and alphanumeric codes used in the species description refer to Kornerup & Wanscher (1966). Microscopic characters were photographed using an Olympus BX50 compound microscope, or an Olympus SZX12 dissecting microscope, with an Evolution MP digital microscope camera operated by ImagePro 6.0. Composite habit photographs were produced using CombineZM (Hadley 2006). Conidiophore morphology was examined from both synnemata on the *Quercus* leaves and cultures grown on MEA.

Phylogeny

DNA was extracted using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories Inc.). ITS barcodes (Schoch et al. 2012) and partial β-tubulin genes were amplified using Illustra pureTaq Ready-To-Go™ PCR Beads (GE Healthcare) with primers ITS1 and ITS4 (White et al. 1990), and Bt2a and Bt2b (Glass & Donaldson 1995). The PCR profile had an initial denaturing step of 95°C for 3 min followed by 35 cycles of 95°C for 1 min, annealing at 56°C for 45 sec and elongation at 72°C for 1 min, followed by a final elongation step at 72°C for 7 min. PCR products were sequenced using the same primers, with the Big Dye Terminator Cycle Premix Kit and products

run on an ABI PRISM™ 310 DNA automated sequencer (Applied Biosystems). Contigs were assembled and edited in CodonCode Aligner v3.7.1.1 (Codon Code Corporation). GenBank accession numbers are included on Fig. 1.

The ITS sequences of the *T. flavovirens* strains were compared to a database of *Talaromyces* spp. compiled from Samson et al. (2011). Reference strains of closely related and other synnema-producing species were included in a more focussed analysis of the β-tubulin gene region. Both data sets were aligned using MAFFT v6.850b (Katoh et al. 2009) using the L-INS-i option. Sequence analysis was done in PAUP*v4.0b10 (Swofford 2002). The BioNJ method was chosen for calculating a single tree for each data set (Gascuel 1997), with *Trichocoma paradoxa* chosen as suitable outgroup for the ITS analysis and a clade of synnema-producing species for the β-tubulin analysis. Bootstrap support was calculated using a 1000 replicates.

Teleomorph-anamorph connection and mating experiments

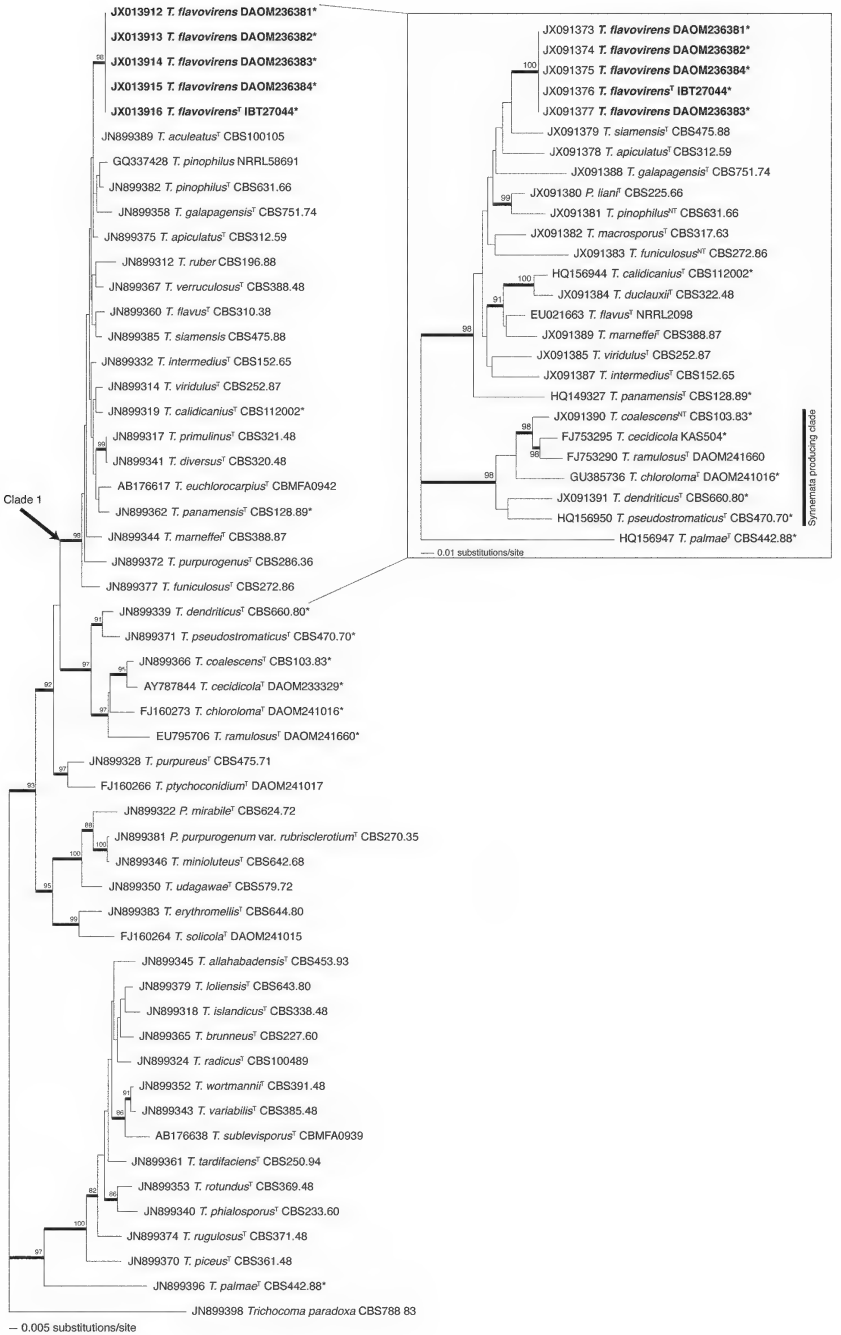
Various approaches were used to prove the connection between the sexual and asexual structures on the newly collected specimens. Cultures were isolated from conidial masses from *Quercus suber* leaves, and ascospore isolates were also attempted.

Anticipating that ascospores might be more heat resistant than conidia, we prepared spore suspensions prepared from both ascomata and synnemata in a semi-solid agar solution (Pitt 1979). Individual suspensions were exposed to temperatures at 5° increments from 50–70°C, for either 15 or 30 min. Unheated suspensions were used as a positive control. After heating, spore suspensions were plated out onto MEA and incubated for 7 days at 25°C, after which plates were inspected for growth.

All cultures were grown on CYA, MEA and Oatmeal agar (OA), and on 2% water agar with twigs or leaves of *Q. macrocarpa* in 9 cm Polystyrene Petri dishes sealed with Parafilm. Further, the strains DAOM 236381–236383 and IBT 27044 were crossed in all possible combinations on OA, and on 2% water agar with *Q. macrocarpa* leaves. After several months of incubation at RT under ambient light, the Parafilm was removed to allow the agar to dry out (López-Villavicencio et al. 2010), in an attempt to mimic the species' arid natural habitat.

Mating-type genes were amplified from all strains using the primers MAT1-1b and MAT1-2b with a touchdown PCR (López-Villavicencio et al. 2010). The PCR profile had an initial denaturing step at 95°C for 5 min, followed by 30 cycles of denaturing at 95°C for 30 sec, annealing starting at 60°C (decreased -0.5°C for every cycle until 50°C) for 30 sec, elongation at 72°C for 1 min, followed by a final elongation step at 72°C for 7 min (López-Villavicencio et al. 2010). To confirm the presence or absence of the MAT1 and MAT2 genes, products were run on a 1% agarose gel. In addition, representative strains containing the putative MAT genes were sequenced to confirm results, using the same sequencing protocol described above. MAT1 and MAT2 sequences were deposited in GenBank with accession numbers JX091392–JX091394.

Figure 1. Phylogenetic trees showing the relationships of *Talaromyces flavovirens* within *Talaromyces*. *Trichocoma paradoxa* was chosen as outgroup in the ITS phylogeny (left). For the β-tubulin phylogeny (right), only close relatives were included in the data set. The phylogeny was rooted to other synnema-producing *Talaromyces* species. Numbers above thick branches represent bootstrap values above 80%. (^{T} = ex-type strain; * = synnemata producer).


JX013912 T. flavovirens DAOM236381*
JX013913 T. flavovirens DAOM236382*
JX013914 T. flavovirens DAOM236383*
JX013915 T. flavovirens DAOM236384*
JX013916 T. flavovirens^T IBT27044*
JN899389 T. aculeatus^T CBS100105
GQ337428 T. pinophilus NRRL58691
JN899382 T. pinophilus^T CBS631.66
JN899358 T. galapagensis^T CBS751.74
JN899375 T. apiculatus^T CBS312.59
JN899312 T. ruber CBS196.88
JN899367 T. verruculosus^T CBS388.48
JN899360 T. flavus^T CBS310.38
JN899385 T. siamensis CBS475.88
JN899332 T. intermedius^T CBS152.65
JN899314 T. viridulus^T CBS252.87
JN899319 T. calidicanius^T CBS112002*
JN899317 T. primulinus^T CBS321.48
JN899341 T. diversus^T CBS320.48
AB176617 T. euchlorocarpius^T CBMFA0942
JN899362 T. panamensis^T CBS128.89*
JN899344 T. marneffei^T CBS388.87
JN899372 T. purpurogenus^T CBS286.36
JN899377 T. funiculosus^T CBS272.86
Clade 1
JN899339 T. dendriticus^T CBS660.80*
JN899371 T. pseudostromaticus^T CBS470.70*
JN899366 T. coalescens^T CBS103.83*
AY787844 T. cecidicola^T DAOM233329*
FJ160273 T. chloroloma^T DAOM241016*
EU795706 T. ramulosus^T DAOM241660*
JN899328 T. purpureus^T CBS475.71
FJ160266 T. ptychoconidium^T DAOM241017
JN899322 P. mirabile^T CBS624.72
JN899381 P. purpurogenum var. rubrisclerotium^T CBS270.35
JN899346 T. minioluteus^T CBS642.68
JN899350 T. udagawae^T CBS579.72
JN899383 T. erythromellis^T CBS644.80
FJ160264 T. solicola^T DAOM241015
JN899345 T. allahabadensis^T CBS453.93
JN899379 T. loliensis^T CBS643.80
JN899318 T. islandicus^T CBS338.48
JN899365 T. brunneus^T CBS227.60
JN899324 T. radicus^T CBS100489
JN899352 T. wortmannii^T CBS391.48
JN899343 T. variabilis^T CBS385.48
AB176638 T. sublevisporus^T CBMFA0939
JN899361 T. tardifaciens^T CBS250.94
JN899353 T. rotundus^T CBS369.48
JN899340 T. phialosporus^T CBS233.60
JN899374 T. rugulosus^T CBS371.48
JN899370 T. piceus^T CBS361.48
JN899396 T. palmae^T CBS442.88*
JN899398 Trichocoma paradoxa CBS788 83
0.005 substitutions/site
JX091373 T. flavovirens DAOM236381*
JX091374 T. flavovirens DAOM236382*
JX091375 T. flavovirens DAOM236384*
JX091376 T. flavovirens^T IBT27044*
JX091377 T. flavovirens DAOM236383*
JX091379 T. siamensis^T CBS475.88
JX091378 T. apiculatus^T CBS312.59
JX091388 T. galapagensis^T CBS751.74
JX091380 P. lianii^T CBS225.66
JX091381 T. pinophilus^NT CBS631.66
JX091382 T. macrosporus^T CBS317.63
JX091383 T. funiculosus^NT CBS272.86
HQ156944 T. calidicanius^T CBS112002*
JX091384 T. duclauxii^T CBS322.48
EU021663 T. flavus^T NRRL2098
JX091389 T. marneffei^T CBS388.87
JX091385 T. viridulus^T CBS252.87
JX091387 T. intermedius^T CBS152.65
HQ149327 T. panamensis^T CBS128.89*
JX091390 T. coalescens^NT CBS103.83*
FJ753295 T. cecidicola KAS504*
FJ753290 T. ramulosus^T DAOM241660
GU385736 T. chloroloma^T DAOM241016*
JX091391 T. dendriticus^T CBS660.80*
HQ156950 T. pseudostromaticus^T CBS470.70*
HQ156947 T. palmae^T CBS442.88*
Synnemata producing clade
0.01 substitutions/site

Results

Isolations from both synnemata and ascomata yielded conidiomata of the asexual state. Because of their small sizes, we could not confidently separate ascospores from conidia under the dissecting microscope, but all attempted ascospore isolations yielded the same synnemata that occurred in conidium-derived isolates. The resulting colonies are described below in the Taxonomy section.

Amplification of ITS barcodes resulted in amplicons about 600 bp long. The aligned data set of *Talaromyces* species was 471 bp long. *Talaromyces flavovirens* was resolved as a subclade of clade1, as described by Samson et al. (2011). Bootstrap supports were low for branches within this clade. Because β-tubulin is very difficult to align across the whole genus, we restricted our analysis of this gene to close relatives identified by the ITS analysis, and we included other synnema-producing species as out groups. β-tubulin amplicons were about 500 bp long, but the confidently aligned portion of the data set was only 391 bp long. Branch lengths were longer than for the ITS phylogeny, but bootstrap support was still low. ITS and β-tubulin analyses resulted in different tree topologies. Although the sister group relationships differed between the two phylogenies, both analyses portrayed *T. flavovirens* as a phylogenetically distinct and coherent species.

No ascomata were observed in uncrossed or crossed cultures of *T. flavovirens*. All strains produced the characteristic yellow, hirsute structures described by Muntañola-Cvetković et al. (2001), but no ascocarp initials or ascospores were observed. Amplification and sequencing of the MAT genes showed that *T. flavovirens* is heterothallic, with DAOM 236382 having the MAT1 gene and other strains possessing the MAT2 gene.

The temperature gradient experiment did not reveal any difference in heat tolerance between ascospores and conidia, and thus was unhelpful in confirming the relationship of the two morphs. In both kinds of spore suspensions, positive controls grew within 7 days. Both ascospores and conidia were killed at 65°C with a 15 min exposure.

Taxonomy

Talaromyces flavovirens (Durieu & Mont.) Visagie, Llimona & Seifert, **comb. nov.**

MycoBank MB800438 Plates 1, 2

≡ *Lasioderma flavovirens* Durieu & Mont., Annls Sci. Nat., Bot., 3e sér., 4: 364. 1845.

Type: Spain, Madrid, Parque del Retiro, on dead leaves of *Quercus ilex*, Nov. 1824, M.C. Durieu de Maisonneuve (**lectotype designated here**, PC 0088796); Spain, Catalonia, Gerona, Selva de Mar, 5.XII.1999, X. Llimona (**epitype designated here**, BCC 473 = BCN 473 ; ex-epitype culture IBT 27044 = CBS 102801).

= *Penicillium aureocephalum* Munt.-Cvetk., Hoyo & Gómez-Bolea, Fungal Diversity 7: 73. 2001.

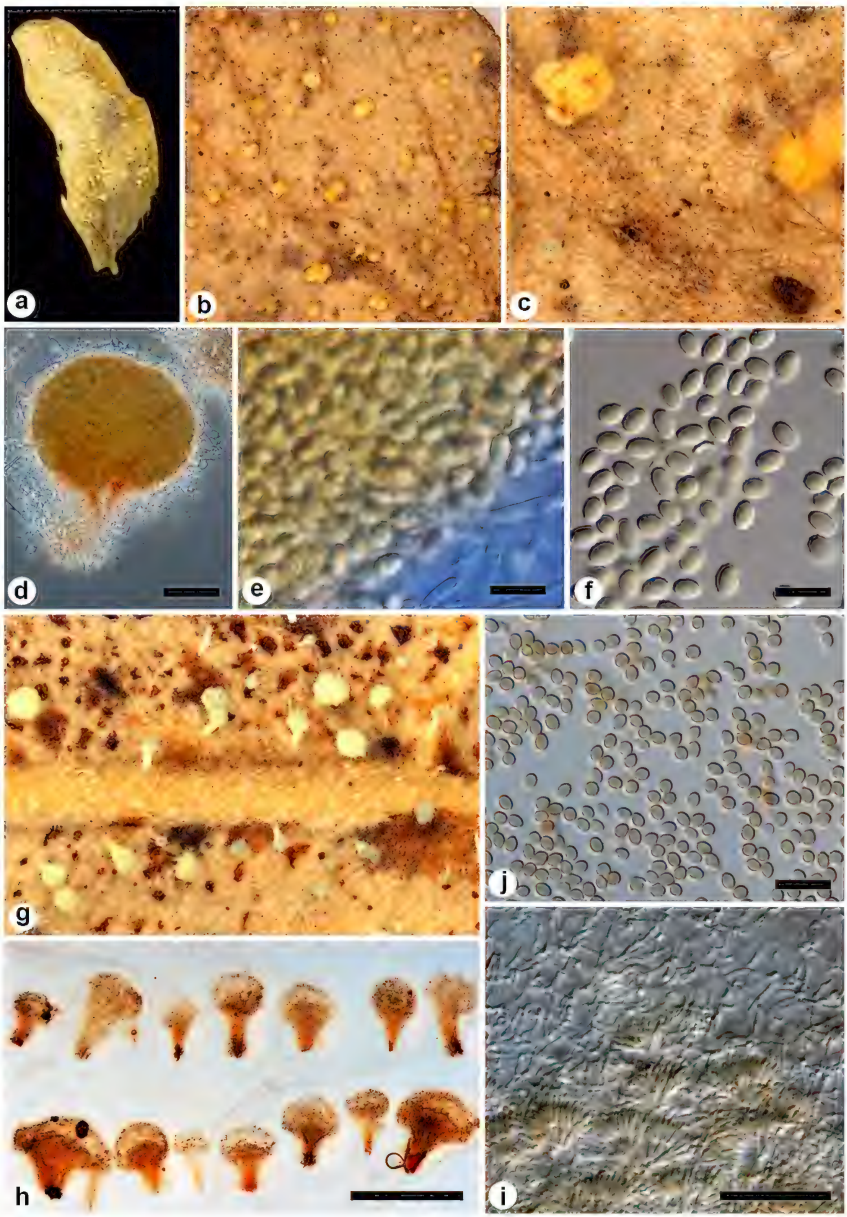


Plate 1. *Talaromyces flavovirens* as it occurs in nature. **a,b.** *Quercus suber* leaves containing the characteristic yellow synnemata and orange gymnothecia of *T. flavovirens*. **c,d.** Gymnothecia on *Quercus suber*. **e,f.** Heavy walled spinose ascospores. **g,h.** Synnemata produced on *Quercus suber*. **i.** Conidiophores in synnema with typical capitulum. **j.** Ellipsoidal heavy walled conidia produced. Scale bars: d = 100 µm; e,f,j = 10 µm; h = 1000 µm; i = 50 µm.

Type: SPAIN, Catalonia, Gerona, Selva de Mar, 5.XII.1999, X. Llimona (holotype of *Penicillium aureocephalum*, BCC 473 = BCN 473; ex-type culture IBT 27044 = CBS 102801)

In nature — Ascomata deep golden yellow, often with a reddish pigment, globose to subglobose, 150–380 µm diam, soft, covered by a loose network of hyphae, with a synnema-like stalk connecting ascomata to leaves, 50–100 × 15–50 µm. Presumed ascocarp initials badly deteriorated, but similar in shape to those of *Talaromyces flavus* and *T. macrosporus* as illustrated by Stolk & Samson (1972), with antheridia coiled around clavate ascogonia. Ascospores abundantly produced, prolate, spiny, with a thick wall that appears double-layered, yellow, (4–)4.5–6(–7) × 3–4, (mean ± stdev = 5.1 ± 0.43 × 3.5 ± 0.2) µm, mean width/length ratio = 0.69 ± 0.06, n = 95. Synnemata similar in appearance to stipitate myxostelid sporangia, with a yellow stalk 400–750 µm high, 100–250 µm wide at the base, and a terminal head 250–300 µm diam comprised of conidiophores and conidia, covered with a bright yellow layer of interwoven sterile hyphae, adding an additional 90–160 µm. Conidiophores mostly biverticillate, with very few subterminal branches occurring, dimensions and other features similar to those described below for cultures, but with metulae consistently diverging at broader angles, perhaps a consequence of the presumably older age of the synnemata on the observed specimens.

In culture — On CYA at 25°C after 7 days — Colonies 11–13 mm, low, planar; texture velutinous; mycelia white; conidiogenesis moderate, conidia dark green (29F6–29F7) in mass; exudate absent, soluble pigment absent; margins narrow (1–2 mm), subsurface, entire; reverse Greyish Green (30D5) under inoculum, Greyish Green (30B3) elsewhere, fading into a Greenish White (30A2) margin. After prolonged incubation, colonies develop a brick red reverse. On MEA at 25°C after 7 days — Colonies 28–31 mm, low, zonate; texture velutinous, appearing almost granular; sometimes dominated by sterile Sulphur Yellow (1A5) mycelia that mask conidial areas; conidiogenesis moderately dense to dense, conidia Dark Green (29F8) in mass; exudate absent, soluble pigment absent; margins wide, 4–6 mm, subsurface, entire; reverse Light Yellow to Reddish Yellow (4A5–4A6) at colony centres, fading to Pale Yellow (2A3–2A4) near margins when aerial mycelia are present, or in colonies lacking aerial mycelium with zonate rings of Greyish Green (30E5) and Greyish Yellow (1B4). After prolonged incubation, colonies produce synnemata similar in appearance to those seen in nature, differing by having shorter stipes; often so densely packed that individual synnemata cannot be distinguished, and conidial heads then seeming confluent. After prolonged incubation, yellow hirsute structures produced, which resemble sterile ascomata. On YES at 25°C after 7 days — Colonies 18–21 mm, low, planar; mycelia white; texture velutinous, with poorly developed funicles; conidiogenesis dense, conidia in

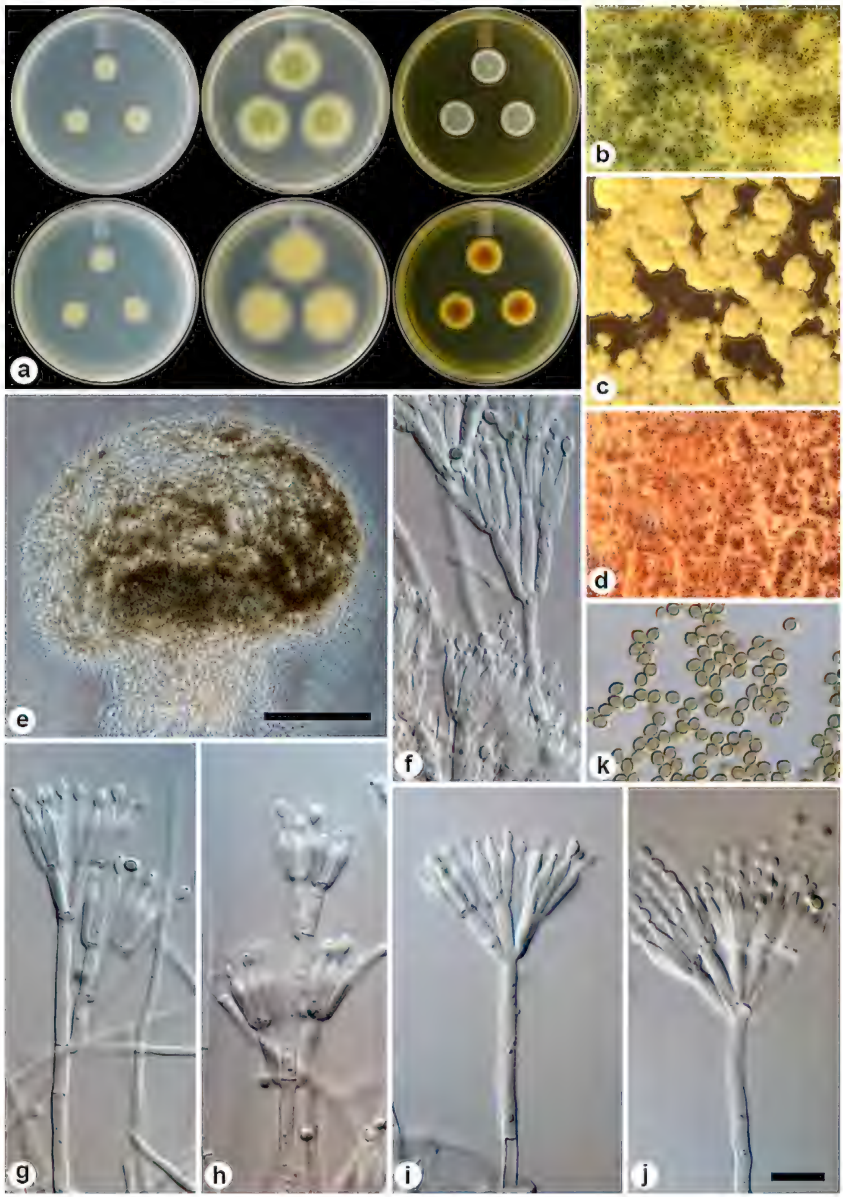


Plate 2. *Talaromyces flavovirens* as it occurs in culture. **a.** Colonies grown for 7 days at 25 °C on CYA, MEA and YES from left to right (top row = obverse, bottom row = reverse). **b.** Colony texture on MEA after 7 days. **c.** Colony texture on MEA after prolonged incubation showing synnemata produced being covered by the sterile yellow capitulum. **d.** Colonies on CYA after prolonged incubation, becoming brick red. **e.** Synnemata produced on MEA after prolonged incubation. **f–j.** Typical conidiophores. **k.** Ellipsoidal heavy walled conidia. Scale bars: e = 50 μm; j (applies to f–k) = 10 μm.

mass Greyish Turquoise (24E4–24E6); exudate absent, soluble pigment absent; margins narrow (3 mm), low, entire; reverse Dark Brown (6F8) at colony centre, fading to Light Brown (6D8) nearing the Greyish Green (30D5) margin, in some strains brown shades lacking, then similarly colored to MEA. On CYA at 5°C after 7 DAYS — no growth. On CYA at 37°C after 7 DAYS — no growth.

Synnemata as described above for the natural substrate. Conidiophores closely packed together, mostly biverticillate although terverticillate branching is not uncommon, smooth walled, 95–325 × 2–3 µm; 2–3 basal branches sometimes present, branches varying greatly in length, 10–35 × 2–3 µm; metulae 4–6 per stipe or branch, cylindrical, forming angles of 25–70°, 9–15 × 2–3 µm. Phialides acerose, in nature with a greenish pigment, in culture pigmentation less obvious, 5–8 per metula, 8–10.5(–11.5) × 2–3 µm. Conidia prolate, rough-walled, connective scars often visible, 2.5–3.5 × 2–2.5 (mean ± stdev = 2.98 ± 0.16 × 2.3 ± 0.13) µm, mean width/length = 0.75 ± 0.05, n = 71, with 'giant' conidia sometimes present, up to 4.5–5.5 × 3.5–4 µm.

HABITAT: Leaves of *Quercus suber, Q. ilex,* and *Cistus salviifolius*.

DISTRIBUTION: Spain, Algeria.

ADDITIONAL SPECIMENS EXAMINED: **SPAIN, CATALONIA,** Port de la Selva, Girona, Font Clareta, on leaves of *Quercus suber,* 3.I.2006, X. Llimona (DAOM 236381, BCN 14.000); Selva de Mar, Girona, 3.I.2006, X. Llimona (DAOM 236382, BCN 14.003); same location, 31.XII.2006, X. Llimona (DAOM 242312); Barraca d'en Rabert, Pau, Girona, 31.XII.2005, X. Llimona (DAOM 236383, BCN 14.006); **VALENCIA,** Xóvar, Alt Palàcia, on leaves of *Q. suber,* 29.XI.2005, X. Llimona, J. Vila, F. Teiedor (DAOM 236384, BCN 14.009). **EXTREMADURA,** Finca Las Cansinas, Monfragüe, Cácera, on leaves of *Q. suber,* 14.XI.1986, R. Galáno no. 507 (DAOM 242311).

Discussion

Talaromyces flavovirens is distinguished from other synnematous *Talaromyces* species (*T. calidicanius, T. chloroloma, T. cecidicola, T. coalescens, T. dendriticus, T. palmae, T. panamensis, T. pseudostromaticus,* and *T. ramulosus*) by the characteristic yellow, short stalked, mycetozoa-like synnemata produced both in nature and in culture. The yellow color is caused by an almost peridium-like hyphal covering of the conidial head, which is much denser than the aerial mycelium that sometimes covers conidiophores of other *Talaromyces* species. Ascomata have so far only been observed in nature, with ascocarps eventually producing thick walled, spiny ascospores. The ecology of *T. flavovirens*, as described by Llimona et al. (2006), is rather specific. The species occurs on dead leaves of *Quercus suber,* rarely *Q. ilex,* and *Cistus salviifolius*, which have not fallen from trees by normal senescence, but rather by physical events such as wildfires, hail or breaking of branches. These leaves are thus killed with their full complement of nutrients, apparently favouring the growth of this fungus. The currently known distribution suggests that this is a moderately thermotolerant, Mediterranean species.

The 1824 specimen from Madrid collected by M.C. Durieu de Maisonneuve (PC 0088796) on leaves of *Quercus ilex* probably represents the Spanish material mentioned by Durieu (1848) and is designated as lectotype of *L. flavovirens* above. The Algerian specimens from la Calle reported by Durieu (1848) were also examined (PC088798, PC0088795), but lack ascomata. It is possible to infer that Montagne (1845) based the description in the protologue on both Algerian and Spanish material, because in the Flore d'Algérie (1848, p. 398), an 1824 collection by Durieu from Madrid is mentioned. Observations of the lectotype by XL demonstrated the occurrence of both ascospores and conidia on this specimen, making it a suitable lectotype for this pleiomorphic species. The alternative, lectotypification using the 1846 illustration, would be less desirable given the lack of conclusive evidence of ascomata. We designated the holotype of *P. aureocephalum* as the epitype of *T. flavovirens* to stabilize the synonymy of these two names.

We were unable to prove unequivocally the genetic connection between the putative asexual and sexual states of *T. flavovirens* but feel that the evidence is convincing. Ascomata were never observed in our single spore derived strains or crossed cultures, although structures that could be interpreted as unfertilized ascomata were sometimes produced. Amplification and sequencing of the mating type genes confirmed that *T. flavovirens* is heterothallic, with individual isolates containing either the MAT1 or MAT2 genes, never both. A recent paper reported the development of a sexual stage for *T. pinophilus* (López-Villavicencio et al. 2010), a close relative of *T. flavovirens,* induced only after media dried out. The similarity with the arid habitat of *T. flavovirens* is interesting, but in our experiments slow drying of the colonies did not induce maturation of the putative ascoma initials.

Comparatively large, prolate ascospores with thick spiny walls were observed, a character also typical for *T. flavus* and *T. macrosporus*. Ascospore sizes were thought to be useful for distinguishing *T. flavus* (3–5 × 2.2–3.5 μm) and *T. macrosporus* (5–6.5 × 3.5–5.2 μm) (Stolk & Samson 1972), but *T. flavovirens* with its broader size range (4–7 × 3–4 μm) complicates this distinction. Based on ascospores alone, *T. flavovirens* is difficult to distinguish from *T. flavus* and *T. macrosporus*, but the distinctiveness of its synnematous morph makes identification simple.

Based on morphological similarities between the sexual states of *T. flavovirens, T. macrosporus,* and *T. flavus*, they were expected to be close relatives in the phylogenetic analyses. *Talaromyces flavovirens* is resolved in clade 1 of Samson et al. (2011), which has little variation in the ITS barcodes. This results in a weak phylogenetic signal, confirming only that *T. flavovirens* itself forms a coherent clade with bootstrap support. The β-tubulin gene has more sequence variation than the ITS and thus provides better species resolution. In the

β-tubulin phylogeny, *T. flavovirens* was resolved as a close relative of *T. apiculatus*[a], *T. galapagensis, T. siamensis*[a], and two small clades containing *Penicillium liani, Talaromyces funiculosus*[a], *T. macrosporus,* and *T. pinophilus*[a]. Amongst these close relatives, are species that lack a known sexual state, marked with [a]. Based on asexual structures, these species are easily distinguished from *T. flavovirens*. Concerning the sexually competent species in this clade, *T. flavus* and *T. macrosporus* are discussed in the previous paragraph. Otherwise, *T. galapagensis* has the most similar ascomata but it produces ascoma initials that are thin hyphae (Samson & Mahoney 1977). Its ascospores, although similarly ornamented, are much bigger (7–10 × 5.5–8 μm) than those of *T. flavovirens*. Other species that produce synnemata are resolved as distant relatives to *T. flavovirens*. Interestingly though, a clade of synnemata producers occurs in the β-tubulin analysis (Fig. 1). This clade occurs in analyses of other genes as well, and some kind of fungus–insect relationship was hypothesized for some of these species (Seifert et al. 2004, Visagie et al. 2009). This idea is being investigated more carefully in our continuing studies of these species.

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Two interesting cantharelloids from Nan and Kanchanaburi Provinces, Thailand

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Abstract —Two cantharelloid fungi are recorded and described from Nan and Kanchanaburi Provinces, Thailand. *Cantharellus atratus*, a new record for Thailand, is recombined in *Craterellus* based on molecular studies. Although previously known from Peninsular Malaysia, *Pterygellus polymorphus* var. *minor* represents a new record for Thailand.

Key words — ectomycorrhizal fungi, ecology, dipterocarps, taxonomy

Introduction

Nan Province is situated in northern Thailand where dipterocarp forests predominate. Several excursions over a two-year period were made to compare macromycetes of a woodland in Veingsa District with a study area in Sisawat District, Kanchanaburi Province, in western Thailand. Two cantharelloids collected during this work are described herein.

Materials & methods

Collections

The Nan locality consisted of seasonal dipterocarp forest dominated by *Shorea obtusa* Wall. and *S. siamensis* Miq. accompanied by *Dipterocarpus tuberculatus* Roxb., *D. obtusifolius* Teijsm. ex Miq. and a major understorey of diverse graminoids. The forest overstorey in Kanchanaburi Province is dominated by *S. siamensis* and *D. tuberculatus* and

an understorey primarily composed of *Cycas siamensis* Miq. and diverse graminoids. The collected specimens were dried in silica gel and deposited in Bangkok, Chulalongkorn University, Microbiology Department (BCUM ND1)

DNA analysis

Genomic DNA extracted from fresh basidiomes with cetyltrimethylammonium bromide (CTAB) as described in Zhou et al. (1999). DNA was amplified in a 50 µl reaction mixture that contained 1 µl of DNA template, 1x buffer [16 mM $(NH_4)_2SO_4$, 67 mMTris-HCl (pH 8.8 at 25°C), 0.01% Tween-20], 2.0 mM $MgCl_2$, 250 µM dNTPs (Bioline Ltd, London, UK), 20 pmol ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990), and 2.5 U BIOTAQ polymerase (Bioline Ltd, London, UK). The PCR cycle comprised an initial denaturation step at 94°C for 5 min followed by 38 cycles of 94°C for 1 min, 51°C for 1 min and 72°C for 1 min, and a final extension at 72°C for 5 min on a TP600TaKaRa PCR Thermal Cycler Dice™ (Takara Bio Inc., Japan). The PCR product was sub-cloned with Takara Cloning Kits (Takara Bio Inc., Japan) following the manufacturer's instructions. Plasmid DNA was extracted from transformed cells suspended in 50 µl sterile water in a 1.5 ml tube that was incubated in boiling water for 5 min. The supernatant was PCR amplified with primers ITS1 and ITS4. After confirming fragment insertion on agarose gels, the inserts were sequenced using Thermo Sequenase Pre-mixed Cycle Sequencing Kits (Amersham International plc. Buckinghamshire, England) using primers Texas Red M13F and T7 following the manufacturer's instructions. The sequences obtained, including the complete ITS regions, were registered in DDBJ. A blast homology search was conducted in DDBJ/EMBL/GenBank database using Blastn. Phylogenetic analysis was performed with Neighbor-Joining method using PAUP 4.08b (Swofford 1999).

SEM observation

Fresh specimens were prepared for the scanning electron microscope by fixation in 2.5% glutaraldehyde 0.1 M phosphate buffer at pH 7.2 for 2 hr at 5°C. Specimens were then rinsed twice in phosphate buffer for 10 min and once in distilled water for 10 min. The specimens were dehydrated in a 30, 50, 70 and 95% ethanol series for 10 min each followed by three changes of absolute ethanol for 10 min each. Specimens were dried in a critical point dryer (Balzers model CPD 020). The dried samples were fixed to brass stubs with double sided sticky tape and then coated with gold in a sputter coater (Balzer model SCD 040). Specimens were observed under SEM (JEOL model JSM 5410LV) with an accelerating voltage of 15 kV. Photographs were recorded by computer.

Taxonomy

Craterellus atratus (Corner) Yomyart, Watling, Phosri, Piapukiew & Sihan., **comb. nov.** FIG. 1

MYCOBANK NO. MB 563344

≡ *Cantharellus atratus* Corner, Ann. Bot. Mem. 2: 62, 1966.

PILEUS 8–9 mm dark fuliginous black, not perforated but depressed at centre, appearing fibrillose-zoned when fresh, centrally stipitate (although limb broader on one side), margin wavy slightly fimbriate. STIPE 7–9 × 0.75–1 mm greyish buff throughout, darker downwards to slightly swollen at base

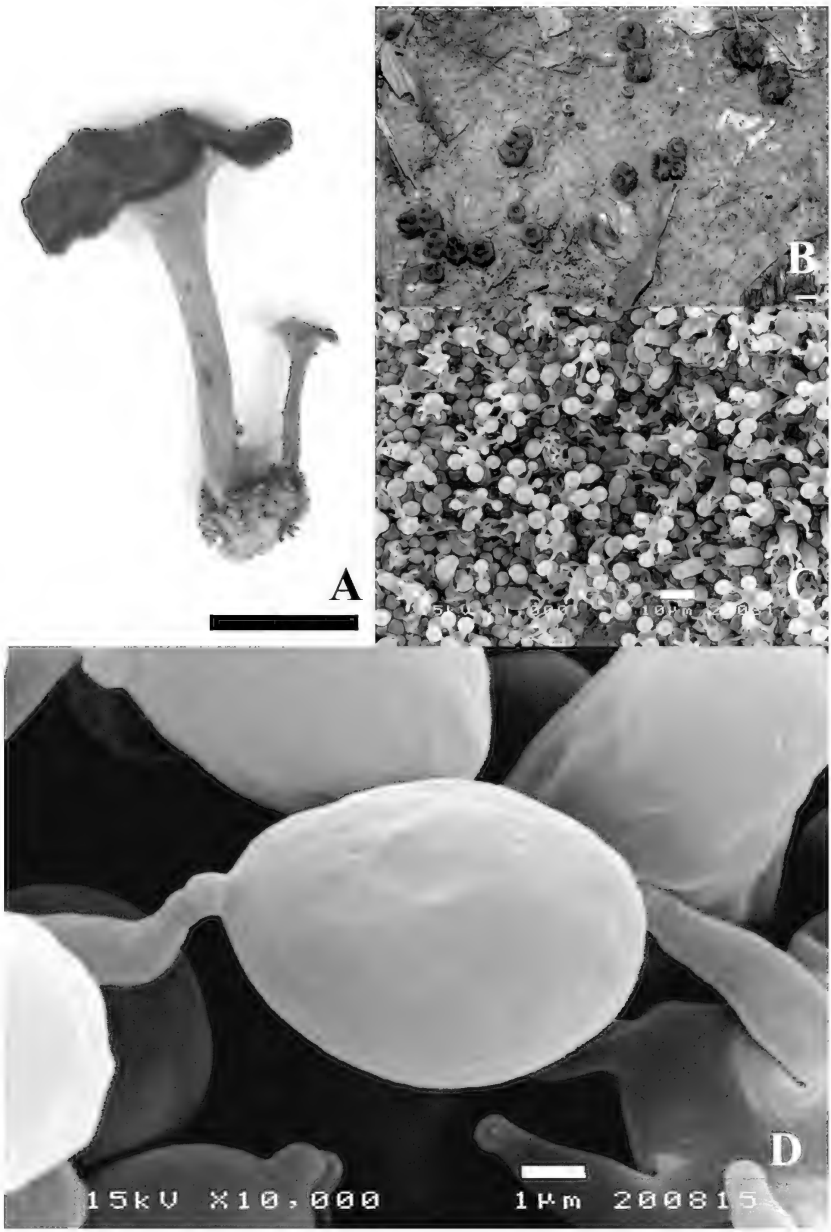
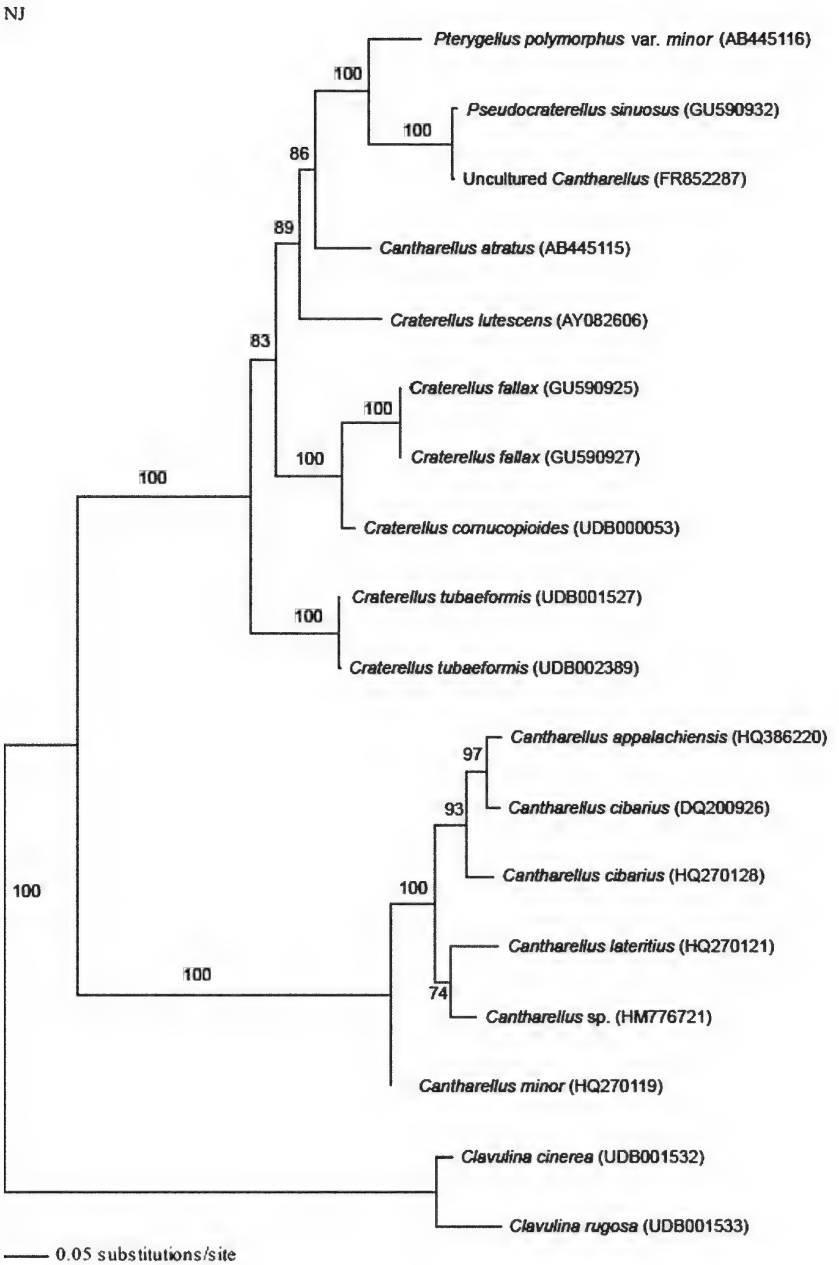


Fig. 1. *Craterellus atratus*. A: Pair of basidiomes. B: Distribution of basidiomes in study area. C: Scanning electron micrograph showing variable number of basidiospores on hymenium. D: High magnification scanning electron micrograph, showing ellipsoid basidiospore shape with smooth wall. Scale bars: A, B = 1 cm; C = 10 µm; D = 1 µm.


NJ
Pterygellus polymorphus var. minor (AB445116)
Pseudocraterellus sinuosus (GU590932)
Uncultured Cantharellus (FR852287)
Cantharellus atratus (AB445115)
Craterellus lutescens (AY082606)
Craterellus fallax (GU590925)
Craterellus fallax (GU590927)
Craterellus cornucopioides (UDB000053)
Craterellus tubaeformis (UDB001527)
Craterellus tubaeformis (UDB002389)
Cantharellus appalachiensis (HQ386220)
Cantharellus cibarius (DQ200926)
Cantharellus cibarius (HQ270128)
Cantharellus lateritius (HQ270121)
Cantharellus sp. (HM776721)
Cantharellus minor (HQ270119)
Clavulina cinerea (UDB001532)
Clavulina rugosa (UDB001533)
100
100
86
89
83
100
100
100
100
97
93
100
100
74
0.05 substitutions/site

embedded in sandy, surface passing irregularly into a pale grey, smooth to very faintly wrinkled hymenium which commences uniformly dark greyish buff but then becomes darker. Flesh concolorous.

Basidiospores 9 × 6–7 µm, ellipsoid, smooth, thin-walled with minute guttules, inamyloid. Basidia 4–6-spored. Cystidia absent. Clamp-connections rare.

Specimen examined: **THAILAND, Nan Province,** Veingsa District, locality, on bare ground in dipterocarp forest, 29 July 2006, coll. Sihanonth (BCMU ND1, GenBank AB445115).

Corner (1966), who described this species from the type collection from Rio de Janeiro, Brazil, also recorded it from Pernambuco, Brazil, and Brunei. Our material agrees with the pickled collection held in Edinburgh as a collection sent by Singer from Pernambuco, Brazil. Singer misidentified the Pernambuco collection as the Venezuelan *Craterellus orinocensis* Pat. & Gaillard, a much larger fungus reaching 8 cm high and 2–3 cm wide, allied with *Cr. cornucopioides* (L.) Pers. (Patouillard & Gaillard 1888) and possibly, as Heinemann (1958) suggests, a variety of *Cr. cornucopioides*. Singer's Pernambuco material is a rather small (pileus 2 cm diam) cantharelloid fungus and resembles a minute, smooth version of the widespread temperate *Cr. tubaeformis* (Fr.) Quél. (= *C. infundibuliformis* (Scop.) Fr.). Unlike *Cr. tubaeformis, Cr. atratus* shows no hint of yellow in the stipe. Corner (1966) originally included *C. atratus* in *Cantharellus* subg. *Phaeocantharellus,* but Donk (1969) indicated that the name *Cantharellus* sect. *Leptocantharellus* Peck has priority for this group. Subsequent molecular studies (Feibelman et al. 1997, Dahlman et al. 2000) have shown that core species in this group are more closely allied to *Craterellus* than to the *Cantharellus cibarius* consortium and should therefore be accepted in *Craterellus* (Fig. 2), and we therefore propose the new combination, *Cr. atratus*.

Pterygellus polymorphus var. ***minor*** Corner, Ann. Bot. Mem. 2: 170, 1966. Fig. 3

Pileus 0.5-3 cm plane or plano-convex & simple at first then almost multipileate from deep incisions, rich, clear chrome-yellow but more ochraceous yellow towards centre in older specimens, deeply depressed, minutely scurfy roughened especially towards margin, smoother inwards and minutely fibrillose; margin uniform when young then soon wavy and flanged, becoming even more so with age and almost dichotomously lobed, irregularly

Fig. 2. A neighbor-joining tree showing placement of *Craterellus atratus* (AB445115) and *Pterygellus polymorphus* var. *minor* (AB445116). *Clavulina cinerea* and *Clavulina rugosa* were used as outgroups. Numerical values on branched are the bootstrap values as percentage bootstrap replication from a 1000 replicate analysis. The scale bar indicates 0.05 of the genetic distance between samples.

Fig. 3. *Pterygellus polymorphus* var. *minor*. A: cluster of basidiomes in study area. B: Scanning electron micrograph showing four basidiospores attached to basidia. C: High magnification scanning electron micrograph of basidiospores, showing smooth wall and ellipsoid shape. Scale bars: A = 1 cm; B = 10 µm; C = 5µm.

incised, wavy and toothed with fimbriate extensions. Stipe rich carrot colour to chrome yellow, darkening downwards, quite short compared with extension of hymenial surface. Hymenium smooth, lacking even minute wrinkles except at pileus margin, whitish or ivory as if frosted, quite deep and irregular and fimbriate, yellow towards basal regions. Flesh thin, pale yellow with a possible faint pinkish cast.

Basidiospores 8–9 × 6–7 µm ellipsoid to broadly ellipsoid, smooth, hyaline, thin-walled, non-amyloid. Basidia 4-spored. Cystidia absent. Clamp-connections absent.

Specimen examined: **THAILAND**, **Kanchanburi Province**, Sisawat District, locality, on soil troops in dipterocarp forest, 20 October 2007, coll. Sihanonth (BCMU KD1, GenBank AB445116).

Although agreeing in all microscopic characters with *P. polymorphus* Corner var. *polymorphus*, the small size of the basidiomes in the Kanchanaburi collections places them in *P. polymorphus* var. *minor,* a small fungus described from the Reservoir Jungle, Singapore, and differing from the type variety in its smaller size, being only slightly over 10 mm maximum. Corner (1966) described the typical variety as growing on soil in woodland in Pahang and Kedah, Malaysia. Our material agrees with both figures and coloured illustrations of *P. polymorphus* var. *minor* as well as with an excellent colour photograph in The Mycologist, portraying material collected in New Guinea (Verbeken & Walleyn 1999). Superficially the fungus approaches *Craterellus aureus* Berk. & M.A. Curtis in outward appearance, except that but the pileus is not perforate.

Our material also resembles *Pseudocraterellus luteus* (Pat.) D.A. Reid, a similar species that Corner (1966) suggested might be compared with *P. polymorphus.* Indeed, all our collections are very close to *Ps. luteus* except for the prominent fimbriate pileus margin present in our material. The basidiome colour also differs, being more yellowish than orange when fresh before finally fading to brownish in *Ps. luteus* whilst *P. polymorphus* is an intense yellow-orange (especially in the stipe) when young before fading to an ochraceous yellow. *Pterygellus* is a stereoid-like fungus and differs from *Pseudocraterellus* in the lack of secondary septation and in the thickened hyphal walls. *Pterygellus polymorphus* sequence analysis places the fungus on a branch close to *Pseudocraterellus sinuosus* (Fr.) Corner, as indicated in Fig. 2.

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Lichens newly recorded from the South Korean coast

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Abstract — Twelve new records of lichen species are reported from the southwestern coast of South Korea. Among them, ten are crustose lichens and two are macrolichens. *Caloplaca yuchiorum* and *Lecidea varians* are reported for the first time from Asia. The coastal rocky area offers great potential for the discovery of new or previously unrecorded crustose lichens in the country.

Key words — biodiversity, taxonomy, maritime, saxicolous, lichenized fungi

Introduction

The southwestern coast of South Korea where we conducted most of our research is mainly composed of rocks surrounded by thousands of small rocky islands (Pl. 1). In contrast to macrolichens, which have been studied thoroughly (Park 1990), crustose lichens are poorly studied in South Korea. Because the rocky coast area harbors a rich diversity of crustose lichens (Joshi et al. 2009, 2011), we focused the study in this area and found many species that have not been reported previously for the country.

Most of the new records reported in this study were growing on coastal rocks, while only a few of them were on bark. Due to the difficulty of collecting lichens on rock, saxicolous lichens are still poorly studied, compared with the terricolous or corticolous ones. In addition, no expert has studied such crustose genera as *Acarospora* A. Massal., *Ochrolechia* A. Massal., or *Pertusaria* DC. in the region, so that future new discoveries might be found within the crustose lichen groups, especially those occurring in the coastal rocky region.

Materials & methods

Specimens were collected from the western and southwestern coast of South Korea in 2011. Samples were hand-sectioned under Nikon SMZ645 dissecting microscope

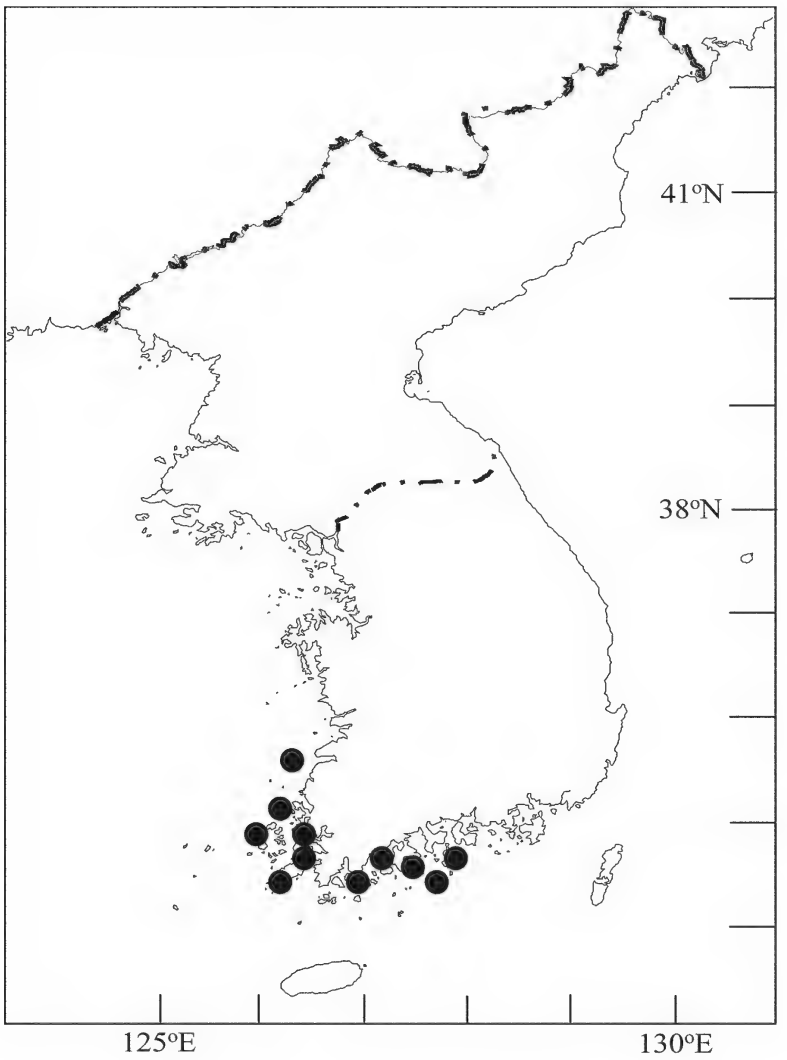

Plate 1. Collection sites for lichens newly recorded from South Korea.

and observed under Nikon E200 microscope using standard microscopical techniques. Lichen substances were identified using standardized thin layer chromatography techniques (TLC) with C system (Orange et al. 2010). The specimens are lodged at herbarium of Lichen & Allied Bioresource Center, Korean Lichen Research Institute (KoLRI), Sunchon National University, Korea.

The new records

Acarospora veronensis A. Massal., Ric. auton. lich. crost.: 29 (1852). Pl. 2A

Thallus medium brown to dark brown, of dispersed areoles, thinly scattered in lines following cracks, or in places as contiguous groups; areoles 0.2–1.0 mm wide, rounded or angular by compression, occasionally slightly lobulate, flat or slightly convex, matt or shiny. Medulla white; lower surface corticated, white to light brown. Apothecia numerous, deeply immersed, round to angular, 0.2–0.4 mm in diam., 1–3 per areole, sometimes confluent, sessile, concave, crater-like, of same color as thallus or reddish brown; epihymenium yellowish brown; hymenium hyaline 60–100 µm high; subhymenium and hypothecium hyaline; Asci cylindrical, more than 100 spores per ascus; Ascospores hyaline, simple, narrowly to broadly ellipsoid, 3–6 × 1.5–2 µm.

Spot tests — thallus: K–, C–, KC–, P–.

Secondary metabolites — none detected.

Specimen examined — **SOUTH KOREA. Gyeongnam:** Namhae county, seaside, 34°43.515′N 127°53.678′E, alt. 16 m, on rock, 28 Apr. 2011, Hur 110244 (KoLRI).

Comments — *Acarospora veronensis* is similar to *A. impressula*, which has more punctiform, less concave apothecia.

Reported from Europe, North & Central America, and Asia (Smith et al. 2009), *A. veronensis* is new to South Korea.

Bacidia egenula (Nyl.) Arnold, Flora 53: 472 (1870). Pl. 2B

Thallus gray-green to yellowish green, crustose, thin to thick. Apothecia flat or slightly convex, 0.2–0.6(–0.8) mm in diam.; disc bluish gray to dull black, epruinose; exciple olive-green at the outer edge, hyaline interior; epihymenium olive-green to blue-green, K–, N+ reddish; hymenium hyaline, colorless, 35–50 µm tall; hypothecium brown to reddish brown in upper part, colorless below. Ascospores hyaline, 3- to 7-septate or indistinctly septate acicular, clavate or long-bacilliform, straight or slightly curved, 25–40 × 1.5–2.5 µm.

Spot tests — thallus: K–, C–, KC–, P–.

Secondary metabolites — none detected.

Specimen examined — **SOUTH KOREA. Gyeongnam:** Namhae county, Changseun Island, 34°51.856′N 127°57.715′E, alt. 4 m, on rock, 29 Apr. 2011, Hur 110289 (KoLRI).

Comments — *Bacidia egenula* is similar to *B. arnoldiana* and *B. viridescens* but *B. arnoldiana* can be distinguished by 1–3-septate spores and a colourless epihymenium and *B. viridescens* differs by its colourless hypothecium and larger thallus granules (80–120 µm diam).

Reported from Europe, North America, and Asia (Smith et al. 2009), *B. egenula* is new to South Korea.

Buellia prospersa (Nyl.) Riddle, Brooklyn Bot. Gard. Mem. 1: 114 (1918). Pl. 2c

Thallus crustose, epilithic, thin to moderately thin, continuous, surface smooth and rimose, slightly roughened, yellowish gray to light gray, epruinose, without soredia or isidia, prothallus absent; medulla white. Apothecia lecideine, 0.5–1.0 mm in diameter, sessile, disc black and plane, epruinose, with black and smooth margin; exciple narrow, inner hyphae hyaline, with darkened outer part; epihymenium brown, hymenium hyaline, paraphyses simple or branched. Asci clavate, *Bacidia*-type, 8-spored, ascospores brown, ellipsoid with obtuse ends, 1-septate, 10–15 × 6–8 µm. Pycnidia not seen.

Spot tests — thallus: K–, C–, KC–, P–.

Secondary metabolites — no compounds or with norstictic acid, connorstictic acid and several xanthones (UV+ orange).

Specimens examined — **SOUTH KOREA. Jeonnam:** Wando county, Cheongsan Island, 34°09.187′N 126°52.821′E, alt. 2 m, 23 Jun. 2011, Hur 110711 (KoLRI); Shinan county, Palgseun Island, 34°47.790′N 126°10.173′E, alt. 1 m, on rock, 2 Jun. 2011, Hur 110389 (KoLRI); Heuksan Island, 34°40.901′N 125°26.671′E, alt. 3 m, 21 Jun. 2011, Hur 110530 (KoLRI).

Comments — *Buellia prospersa* is easily separated from other Korean *Buellia* species by its yellowish surface (UV+ orange) and growth only on the coastal siliceous rocks.

Cosmopolitan but restricted to coastal areas (Nash et al. 2007), *B. prospersa* is new to South Korea.

Caloplaca yuchiorum Lendemer & C.A. Morse, J. Torrey Bot. Soc. 137: 328 (2010). Pl. 2d

Thallus gray-white, rimose-areolate, areoles plane, smooth, upper surface postulate, and pustules broken and forming soredia; prothallus black. Apothecia immersed to subimmersed, up to 0.7 mm, with only proper margin or with additional thalloid margin, proper margin black when prominent; disc dark brown to black, flat; exciple with or without algae; epihymenium light brown with blue-green, K+ light violet, N+ violet; hymenium 65–85 µm, with oil droplets; hypothecium hyaline, with oil droplets. Ascospores hyaline, 2 locules, ellipsoid, (9–)11–18 × (4–)4.5–7 µm, isthmus at least 1/3 of spore length. Pycnidia present, immersed, conidia bacilliform, 3.5–4.5 × 1–1.2 µm.

Spot tests — cortex: K+ yellow, C–, N–; medulla: K–, C–, N–.

Secondary metabolites — atranorin (thallus), anthraquinone pigment (epihymenium).

Specimens examined — **SOUTH KOREA. Gyeongnam:** Namhae county, Changseun Island, 34°51.891′N 127°57.705′E, alt. 2 m, on rock, 29 Apr. 2011, Hur 110280 (KoLRI); Tongyeong city, Salyang Island, 34°50.590′N 128°12.137′E, alt. 28 m, on rock, 20 Apr. 2011, Hur 110028 (KoLRI). **Jeonam:** Jindo county, seaside, 34°32.910′N 126°18.697′E, alt. 3 m, on rock, 3 Jun. 2011, Hur 110462 (KoLRI).

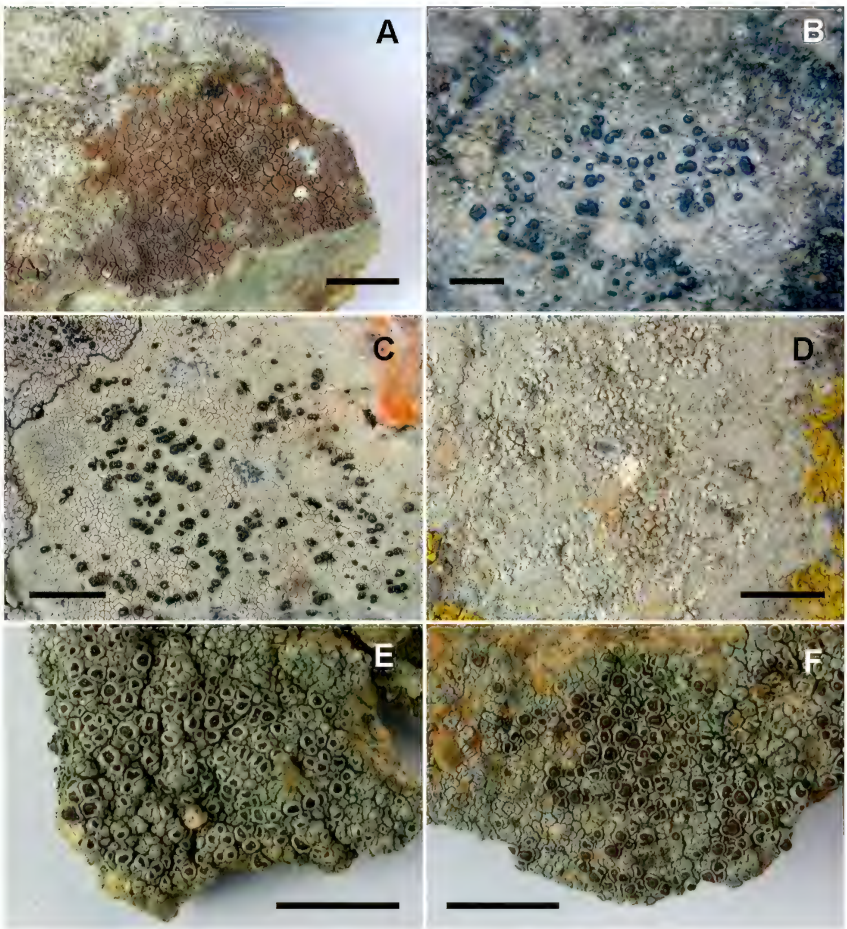

Plate 2. Habit of newly recorded lichens. A. *Acarospora veronensis*, Hur 110244; B. *Bacidia egenula*, Hur 110289; C. *Buellia prospersa*, Hur 110598; D. *Caloplaca yuchiorum*, Hur 110284; E. *Lecanora melacarpella*, Hur 110352; F. *Lecanora pseudistera*, Hur 110466. Scale bars: B = 1 mm; A, C–F = 5 mm.

Comments — *Caloplaca yuchiorum* is very similar to *C. diplacia*, which differs in having a sorediate thallus with a pale prothallus, smaller apothecia (0.3–1.0 mm wide), and an epihymenium with an anthraquinone pigment that reacts K + magenta.

Reported from North America (Lendemer & Morse 2010), *C. yuchiorum* is new to South Korea and Asia.

Lecanora melacarpella Müll. Arg., Bull. Herb. Boissier 3: 633 (1895) Pl. 2E

Thallus whitish to grayish white, crustose, uniform, continuous or dispersed verrucose to verruculose, epruinose; margins indefinite or definite, sometimes with a whitish prothallus. Apothecia sessile, constricted at base, 0.6–1.5 mm diam.; discs brown to dark brown, epruinose; margins smooth to verruculose or verrucose, prominent. Amphithecium with small K–soluble and large crystals; parathecium hyaline with small K–soluble crystals; epithecium without crystals, with a 2 µm high hyaline layer above; hymenium hyaline, 65–85 µm tall; hypothecium hyaline, inspersed with oil droplets. Ascospores ellipsoid, (9–)10.5–13.5 (–15.5) × (5.5–) 7.5–8.5 µm.

Spot tests — thallus: K+ yellow, C–, KC–, Pd+ light orange.

Secondary metabolites — atranorin, zeorin, unidentified triterpenoid (minor).

Specimens examined — **SOUTH KOREA. Gyeongnam:** Namhae county, Mt. Mangeun, 34°51.076′N 127°49.605′E, alt. 165 m, 29 Apr. 2011, Hur 110144 (KoLRI). **Jeonam:** Jindo county, Jeob-do Island, 34°23.091′N 126°18.250′E, alt. 2 m, 3 Jun. 2011, Hur 110516 (KoLRI); Shinan county, Palgeum Island, 35°47.734′N 126°10.219′E, alt. 2 m, 2 Jun. 2011, Hur 110352 (KoLRI).

Comments — *Lecanora melacarpella* is similar to *L. gangaleoides*, which is diagnosed by gangaleoidin and a thicker, verrucose thallus.

Reported from Australasia and India (Lumbsch & Elix 2004, Galloway 2007), *L. melacarpella* is new to South Korea.

Lecanora pseudistera Nyl., Flora 55: 354 (1872). Pl. 2F

Thallus yellowish gray or whitish gray, crustose to subsquamulose, surface smooth and epruinose, without soredia, verrucose-areolate or dispersed-areoles. Apothecia 0.4–1.5(–2.0) mm in diam., red-brown to brown, plane, epruinose, subimmersed to sessile; margins concolorous with thallus, thin or thick, persistent, entire or flexuose, even and entire; amphithecium with large crystals insoluble in K; epithecium without crystals, red-brown to orange-brown; hymenium hyaline, c. 60 µm high, paraphyses with a clear cap; subhymenium hyaline, c. 20 µm. Ascospores, hyaline, simple, ellipsoid, 9–15 × 5–7 µm. Pycnidia not seen.

Spot tests — thallus: K+ yellow, C–, KC–, P+ light orange.

Secondary metabolites — atranorin, 2'-O-methylperlatolic acid.

Specimen examined — **SOUTH KOREA. Jeonam:** Jindo county, seaside, 34°32.910′N 126°18.697′E, alt. 3 m, on rock, 2 Jun. 2011, Hur 110461, 110466 (KoLRI).

Comments — *Lecanora pseudistera* is very similar to *L. campestris*, which has small crystals in the amphithecium and lacks 2'-O-methylperlatolic acid.

Reported from Europe, the Americas, Asia, South Africa, and Australasia (Lumbsch & Elix 2004), *L. pseudistera* is new to South Korea.

Lecanora strobilina (Spreng.) Kieff., Bull. Soc. Hist. Nat. Metz 19: 72 (1895). Pl. 3A

Thallus greenish gray to yellowish gray, crustose, without soredia, poorly developed or granular-warted with areoles or rimose; areoles convex, 0.1 – 0.2 mm in diameter; Apothecia rounded or slightly irregular in outline, rarely single, mostly densely crowded, 0.30–0.40 mm in diam.; disc yellowish ochre to orange-brown, flat to moderately or rarely strongly convex, dull, finely white pruinose; margin white, pale gray or more rarely of same color as the thallus. Amphithecium present, with an algal layer filled with small granules; epihymenium orange-brown; hymenium hyaline, (35–)40–50 µm tall; subhymenium hyaline. Ascospores hyaline, simple, narrowly ellipsoid, (8.5–)10.6–12.4(–16.5) × (3–)3.3–3.8(–4.5) µm.

Spot tests — thallus: K–, C–, KC–, P–.

Secondary metabolites — usnic acid, decarboxysquamatic acid, ± zeorin.

Specimen examined — **SOUTH KOREA. Jeonam:** Jindo county, Jeob-do Island, 34°23.091′N 126°18.250′E, alt. 2 m, on the *Pinus* bark from the coastal region, 3 Jun. 2011, Hur 110513 (KoLRI); Hachodo Island, 34°16.592′N 126°04.215′E, alt. 22 m, on the *Pinus* bark from the coastal region, 23 Aug. 2011, Hur 110854 (KoLRI); Shinan county, Bogil Island, 34°09.275′N 126°34.740′E, alt. 3 m, on the *Pinus* bark from the coastal region, 23 Jun. 2011, Hur 110635 (KoLRI); Palgeum Island, 35°47.790′N 126°10.173′E, alt. 1 m, on the *Pinus* bark from the coastal region, 2 Jun. 2011, Hur 110392 (KoLRI).

Comments — *Lecanora strobilina* is very similar to *L. confusa* but distinguished by absence of xanthones and lower hymenium (not exceeding 70 µm).

Reported from Europe, Macaronesia, North America, Asia, and Africa (Smith et al. 2009), *L. strobilina* is new to South Korea.

Lecidea varians Ach., Syn. Meth. Lich.: 38 (1814). Pl. 3B

Thallus grayish green to yellowish green, thin, without soredia, mostly continuous, sometimes warted-aerolate, rimose or rarely endosubstratal thallus. Apothecia small and rounded, 0.15–0.4 mm in diam., often in groups of 3–5, sessile; disc brown to reddish brown, with or without white pruina, plane to weakly convex; margin weakly prominent and soon excluded or lacking from the beginning; exciple brown at the outer edge, hyaline interior; epihymenium brown; hymenium hyaline, 50–70 µm high, subhymenium and hypothecium hyaline. Asci *Lecanora*-type, ascospores hyaline, simple, ellipsoid to broadly ellipsoid, 8–13 × (4–)5–7 µm.

Spot tests — thallus: K+ yellow, C–, P–, UV+ orange.

Secondary metabolites — atranorin, xanthones.

Specimen examined — **SOUTH KOREA. Gyeongnam:** Tongyeong city, Salyang Island, 34°51.045′N 128°12.364′E, alt. 45 m, on the *Prunus* bark from the coastal region, 20 Apr. 2011, Hur 110035 (KoLRI).

Comments — Although *L. varians* does not belong to *Lecidea* s. str., we retain it here because its generic position is still unclear.

Reported from North America and Europe (Nash et al. 2004), *L. varians* is new to South Korea and Asia.

Ochrolechia tartarea (L.) A. Massal., Ric. auton. lich. crost.: 30 (1852). Pl. 3c

Thallus pale to dark gray, often very thick, to 3 mm or more, surface soft, powdery-tartareous, often with numerous irregular warts forming an uneven corrugate crust, sometimes with a paler zoned margin and a pale prothallus. Apothecia usually frequent, immersed and closed at first, becoming rounded or irregular, scattered or crowded, sessile; thalline exciple thick, wavy; disc to 5 mm diam., pale brown to dull orange-pink, concave to flat, the surface often scabrose-roughened; epihymenium granular, the granules dissolving in K; hymenium 200–300 µm; hypothecium light yellow. Ascospores (30–) 40–70 × 20–40 µm, broadly ellipsoid.

Spot tests — cortex and medulla: K–, C+ pink, KC+ red, Pd–; apothecial disc C+ red.

Secondary metabolites — gyrophoric acid, lecanoric acid.

Specimens examined — **South Korea**. Gyeongnam: Namhae county, Mt. Daebang, 34°51.006′N 127°59.427′E, alt. 87 m, on rock, 20 Apr. 2011, Hur 110274 (KoLRI). **Jeonam**: Wando county, Cheongsan Island, 34°09.187′N 126°52.821′E, alt. 2 m, on rock, 23 Jun. 2011, Hur 110733 (KoLRI).

Comments — Its tartareous C+ pink thallus distinguishes *O. tartarea* from the morphologically similar *O. parella,* which produces a thallus that is fissured and C + yellow.

Reported from Europe, Macaronesia, North America, Asia, Africa, and subantarctic (Galloway 2007, Smith et al. 2009), *O. tartarea* is new to South Korea.

Pertusaria flavicans Lamy, Bull. Soc. bot. Fr. 25: 427 (1878). Pl. 3d

Thallus crustose, epilithic, rather thick, surface roughened, irregularly coarsely warted, usually continuous, slightly rimose to areolate, yellowish gray to light yellowish; areoles irregular in shape or rounded, flat or sometimes slightly convex, ≤1 mm in diameter, sometimes papillae present; prothallus absent; yellow soralia on the surface, abundant, 0.5–1 mm in diameter, often gathered in the central part of the thallus, color paler than the thallus, coarsely granular; isidia absent. Apothecia and pycnidia not seen.

Spot tests — thallus: K–, C+ orange, KC+ orange, P–.

Secondary metabolites — norstictic acid, salazinic and xanthones (UV+ orange).

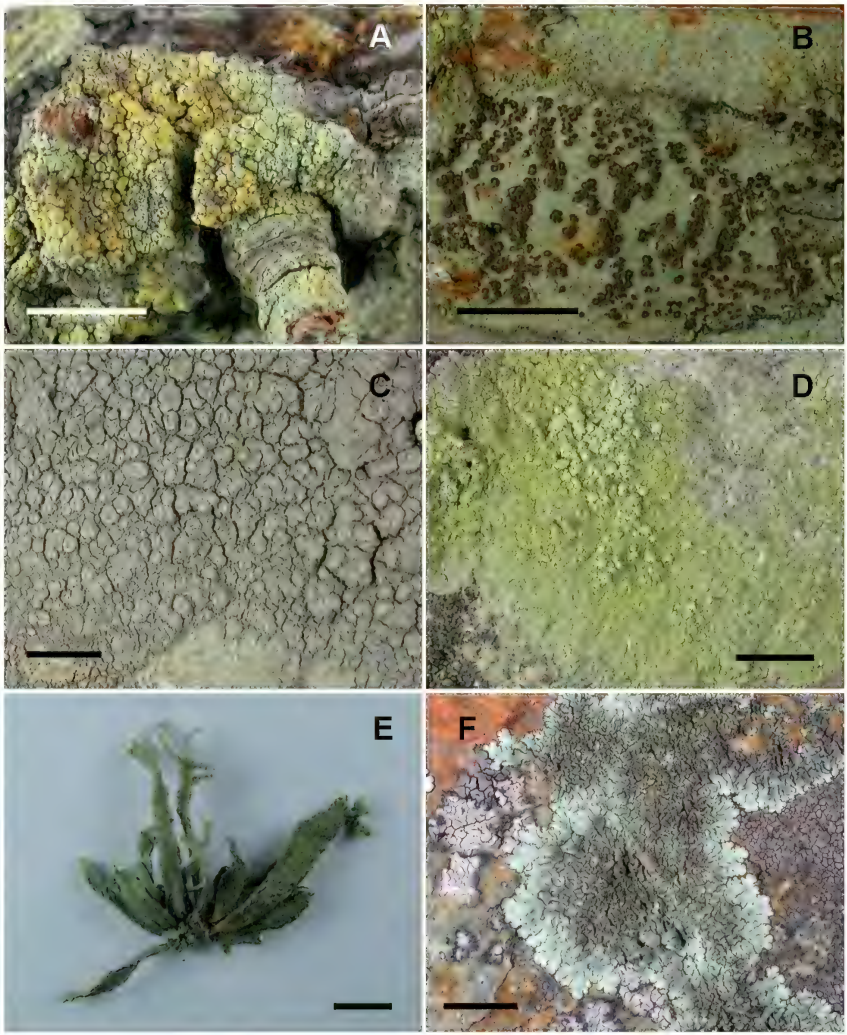

Plate 3. Habit of newly recorded lichens. A. *Lecanora strobilina*, Hur 110513; B. *Lecidea varians*, Hur 110035; C. *Ochrolechia tartarea*, Hur 110274; D. *Pertusaria flavicans*, Hur 110259; E. *Ramalina sekika*, Hur 110645; F. *Xanthoparmelia congensis*, Hur 110949. Scale bars = 5 mm.

Specimens examined — **SOUTH KOREA. Gyeongnam**: Tongyeong city, Salyang Island, 34°51.016′N 128°11.985′E, alt. 300 m, on rock, 20 Apr. 2011, Hur 110050 (KoLRI). **Jeonam**: Wando county, Cheongsan Island, 34°12.218′N 126°54.357′E, alt. 5 m, on rock, 23 Jun. 2011, Hur 110757 (KoLRI). **Jeonbuk**: Gunsam city, Shinsi Island, 35°49.091′N 126°28.159′E, alt. 141 m, on rock, 22 Aug. 2011, Hur 110780 (KoLRI).

Comments — *Pertusaria flavicans* is easily recognized by its yellowish (UV+ orange) thallus, yellow soralia, and growth only in the coastal rocks.

Reported from Europe, Macaronesia, and Asia (Smith et al. 2009), *Pertusaria flavicans* is new to South Korea.

Ramalina sekika Asahina, Journ. Jap. Bot. 17: 138 (1941). Pl. 3E

Thallus growing on the rock, fruticose, erect and forming tufts, attached by a basal holdfast, surface dull yellowish green or grayish green, turning brownish near the base, 1–3 cm long, with a few branches; branch 2–5 mm wide, flattened and solid, wider in the middle part, irregularly branched near the tips; surface rather scabrid, covered with numerous pseudocyphellae surrounded by granules, the other side of the surface often smooth and yellowish; cortex thin, composed of thick-walled hyphae, medulla white; Apothecia and pycnidia not found.

Spot tests — cortex: K–, C–, KC+ yellow; medulla: K–, C–, KC+ pale red.

Secondary metabolites — usnic, sekikaic, 4'-O-demethylsekikaic and ±salazinic acids.

Specimens examined — **SOUTH KOREA. Gyeongnam:** Namhae county, seaside, 34°48.853′N 127°49.698′E, alt. 2 m, on rock, 28 Apr. 2011, Hur 110219 (KoLRI). **Jeonam:** Yeonggwang county, West seaside, 35°19.635′N 126°22.695′E, alt. 2 m, on rock, 1 Jun. 2011, Hur 110299 (KoLRI); Wando county, Cheongsan Island, 34°09.187′N 126°52.821′E, alt. 2 m, on rock, 23 Jun. 2011, Hur 110707 (KoLRI).

Comments — *Ramalina sekika* usually grows together with *R. pollinaria*; both species have similar habitat and morphology, but *R. pollinaria* has fine soralia on the surface and marginal part, while *R. sekika* has rough surface covered with pseudocyphellae and usually contains salazinic acid.

Reported from China and Japan (Asahina 1941, Wei 1991), *Ramalina sekika* is new to South Korea.

Xanthoparmelia congensis (J. Steiner) Hale, Phytologia 28: 486 (1974). Pl. 3F

Thallus foliose, tightly adnate on the rock, 2–5 cm broad, dark yellowish green in the central part, turning light yellow-green near the tips; lobes rather narrow, sublinear, 0.2–0.6 mm wide, sometimes branched, tips subrounded to rounded; upper surface shiny, covered with dense globose isidia, ≤0.1 mm in diameter, paler than the thallus, mainly in the central part of the thallus, tip part sometimes bursting open but not becoming sorediate, without any branches; medulla white; lower surface black and shiny, plane and smooth, black rhizines sparse, simple, c. 0.3 mm long; Apothecia and pycnidia not seen.

Spot tests — cortex: K–, C–, KC–, P+ orange; medulla: K+ yellow to red, C–, KC–, P+ orange.

Secondary metabolites — stictic acid (major), constictic, cryptostictic, norstictic, menegazziaic and usnic acid.

Specimens examined — **SOUTH KOREA. Jeonam:** Gohong county, Wenaro Island, 34°27.236′N 127°31.153′E, alt. 8 m, on rock, 24 Aug. 2011, Hur 110219 (KoLRI). Shinan county, Heuksan Island, 34°39.882′N 125°26.189′E, alt. 5 m, on rock, 21 Jun. 2011, Hur 110557 (KoLRI).

Comments — Compared with other Korean *Xanthoparmelia* species, *X. congensis* is relatively small in size and found only in the seaside rocks. It might be confused with *X. orientalis,* which also has the black lower surface, which is distinguished by its larger lobes (1–2. 5 mm) and different chemical compounds (usnic acid, salazinic acid, consalazinic acid, and trace of norstictic acid).

Reported from Mexico, South Africa, Australia, India and China (Hale 1990), *Xanthoparmelia congensis* is new to South Korea.

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Polyporus submelanopus sp. nov. (*Polyporales, Basidiomycota*) from Northwest China

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Abstract — *Polyporus submelanopus*, collected from Qinghai Province, Northwest China, is described and illustrated based on both morphological and phylogenetic analyses. It is characterized by a terrestrial habit, black-stipitate basidiocarps, circular to angular pores, two kinds of generative hyphae bearing simple septa and clamp connections respectively, and cylindrical smooth hyaline basidiospores. Morphologically, *P. submelanopus* belongs to the melanopus group and resembles *P. melanopus* in sharing similar pilei with a shallow central depression, circular to angular pores, basidiospores, and a terrestrial habit. However, *P. melanopus* has smaller pores, thicker and entire dissepiment, fusoid cystidioles, and clamped generative hyphae only. The differences between *P. submelanopus* and *P. badius, P. xinjiangensis,* and *P. tubaeformis* are discussed as well. Phylogenetic ITS sequence analysis supports *P. submelanopus* within the melanopus group but distinctly separate from other sampled species of this group.

Key words —*Polyporaceae,* polypore, taxonomy, wood-inhabiting fungi

Introduction

Polyporus P. Micheli ex Adans., type genus of the *Polyporaceae,* is a well-known polypore genus. It is characterized by typical formation of stipitate to substipitate basidiocarps, a dimitic hyphal system with arboriform skeleto-binding hyphae, smooth cylindrical to subellipsoid inamyloid basidiospores, and causing a white rot (Gilbertson & Ryvarden 1987, Núñez & Ryvarden 1995).

Núñez & Ryvarden (1995) divided all 32 species of *Polyporus* into six morphological groups without taxonomic rank: Admirabilis, Dendropolyporus [= *Dendropolyporus* (Pouzar) Jülich], Favolus (= *Favolus* Fr.), Polyporellus (= *Polyporellus* P. Karst.), Melanopus (= *Melanopus* Pat.), and Polyporus. The melanopus group is characterized by coriaceous and black-stipitate

basidiocarps, skeleto-binding hyphae mostly with narrow lumen but solid when mature, medium to large basidiospores (6–12 × 2–4 μm), and growth on dead woods except for the terrestrial *Polyporus melanopus* (Pers.) Fr. Since Núñez & Ryvarden (1995), some species have been newly described and/or emended based on morphology (Dai et al. 2003, 2007c, 2009; Sotome et al. 2007; Dai 2012a) or combined morphological and phylogenetic evidence (Sotome et al. 2011).

China is a vast area with varied landscapes and forests inhabited by a high diversity of wood-decaying fungi. More than 1,200 poroid, corticioid, and hydnoid taxa have been recorded in China (Dai 2011, 2012b). *Polyporus* species have been repeatedly documented from different parts of the country (Dai et al. 2003, 2004, 2007a,b,c,d, 2009; Dai & Penttilä 2006; Cui et al. 2008; Li et al. 2008; Yuan & Dai 2008; Wang et al. 2011), with 36 total species recorded before this study (Dai 2012b). However, many specimens deposited at the herbarium of Institute of Applied Ecology, Chinese Academy of Sciences (IFP), are still not identified to species level. During the re-examination of these specimens, we found two that match the *Polyporus* concept but were distinct from any known species based on both morphological and phylogenetic analyses. They are described and illustrated here as *Polyporus submelanopus*.

Materials & methods

The studied specimens are deposited at IFP, China, and Botanical Museum, Finnish Museum of Natural History (H), Helsinki. The microscopic procedure follows Dai (2010). Sections were studied at magnifications up to ×1000 using a Nikon Eclipse 80i microscope and phase contrast illumination. Measurements are made from sections stained with Cotton Blue. In this text, CB stands for Cotton Blue, CB+ for cyanophilous, CB– for acyanophilous, IKI for Melzer's reagent, IKI– for negative in Melzer's reagent, and KOH for 5% potassium hydroxide. In presenting the variation in the size of the basidiospores, 5% of measurements were excluded from each end of the range and given in parentheses. The meanings of other abbreviations are as follows: L = mean basidiospore length (arithmetic average of all basidiospores), W = mean basidiospore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios between the specimens studied, and n = number of basidiospores measured from given number of specimens. Drawings are made with the aid of a drawing tube. Special color terms follow Anonymous (1969) and Petersen (1996).

ITS sequences were obtained from herbarium specimens using Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland). The primers ITS5 and ITS4 (White et al. 1990) were used to perform PCR amplifications and subsequent sequencing reactions. Four newly generated sequences were deposited at GenBank (accession numbers JQ964422–JQ964425). Other ITS sequences were downloaded from GenBank (Table 1). *Polyporus grammocephalus* Berk. and *P. pseudobetulinus* (Murashk. ex Pilát) Thorn et al. were selected as outgroup (Krüger et al. 2006). The procedure of phylogenetic analysis was as below. Firstly, nucleotide sequences were aligned using ClustalX 2.0 (Larkin et al. 2007)

Table 1. ITS sequences from *Polyporus* used in the phylogenetic analysis

Species	Voucher No.[a]	Origin	GenBank No.
P. badius	–	Japan	AB587625
	–	USA	AF516558
	–	USA	AF516559
P. guianensis Mont.	–	Argentina	AF516564
	–	Venezuela	AF516566
P. leprieurii Mont.	–	Costa Rica	AF516567
P. melanopus	–	Argentina	AF516568
	–	Argentina	AF516569
	–	Austria	AF518759
	H 6003449	Finland	JQ964422
	H 6029190	Finland	JQ964423
P. mikawai Lloyd	–	China	AF516570
P. submelanopus	IFP 004953	China	JQ964424
— (holotype)	IFP 004946	China	JQ964425
P. tubaeformis	–	UK	AF511438
	–	UK	AF511439
	–	USA	AF511440
	–	USA	AF511441
	–	USA	AF511442
	–	Norway	AF511443
P. varius (Pers.) Fr.	–	Germany	AF516574
	–	Germany	AF516575
	–	Russia	AF516576
	–	USA	AF516577
	–	USA	AF516578
	–	USA	AF516579
	–	USA	AF516580
	–	Japan	AB587636
P. grammocephalus	–	Japan	AB587626
P. pseudobetulinus	–	Japan	AB587644

[a] Provided only for specimens newly sequenced in this study.

with defaulted parameters; secondly, the best-fit evolutionary models for maximum likelihood (ML) analysis and Bayesian analysis were estimated by jModelTest (Guindon & Gascuel 2003, Posada 2008) based on corrected Akaike information criterion; finally, following the optimal models, maximum likelihood (ML) and Bayesian trees were constructed using PhyML 3.0 (Guindon & Gascuel 2003) and Mrbayes3.2 (Ronquist et al. 2012), respectively. The ML tree was tested with 100 bootstrap replicates. For Bayesian analysis, two independent rums were employed. Each run performed a Metropolis-coupled Markov chain Monte Carlo analysis with four chains for 1,000,000 generations. Trees were saved per 100th generation. The value of burn-in was set to discard the first 25% trees, and other trees were used to compute a 50% consensus tree and calculate Bayesian posterior probability.

Taxonomy

Polyporus submelanopus H.J. Xue & L.W. Zhou, **sp. nov.** FIG. 1

MycoBank MB 800237

Differs from *Polyporus melanopus* in having two kinds of generative hyphae (one with simple septa and one with clamp connections), slightly larger pores, thinner and lacerate dissepiments, and no fusoid cystidioles.

TYPE: China, Qinghai Province, Huzhu County, Beishan Forest Farm, on ground in forest of *Picea*, 1.IX.2003 Dai 5015 (**holotype** IFP 004946; GenBank sequence JQ964425).

ETYMOLOGY: *submelanopus* (Lat.): somewhat similar to *Polyporus melanopus*.

BASIDIOMES: Basidiocarps annual, terrestrial, centrally or laterally stipitate, solitary. Pilei sometimes slightly infundibuliform, up to 6.2 cm in diam, and 8 mm thick. Pileal surface cinnamon-buff to vinaceous, glabrous, sometimes wrinkled and faintly zonate when dry; margin acute, concolorous with pileus, curved down when dry. Pore surface straw-yellow to honey-yellow; pores circular to angular, 2–3 per mm; dissepiments thin, lacerate. Context white to cream, soft corky when dry, up to 6 mm thick. Tubes cream to straw-yellow, decurrent on about three fifths of one side of the stipe with obviously boundary, up to 2 mm long. Stipe bearing a fuscous to black cuticle, corky, up to 7 cm long, and 8 mm in diam.

HYPHAL STRUCTURE: Hyphal system dimitic; two kinds of generative hyphae with one type bearing only simple septa and the other with clamp connections at each septum; skeletal hyphae arboriform, IKI–, weakly CB+; tissue unchanged in KOH.

CONTEXT: Generative hyphae common, thin-walled, occasionally branched, 3.5–4 μm in diam; arboriform skeletal hyphae frequent, thick-walled with a wide lumen, moderately branched, aseptate, interwoven, up to 7 μm in diam.

TUBES: Generative hyphae frequent, hyaline, thin-walled, frequently branched and simple septate, clamp connection frequent on the generative hyphae near the basidia, 1.5–3.5 μm in diam; arboriform skeletal hyphae common, thick-walled with a wide to narrow lumen, moderately branched, aseptate, interwoven, 1.5–3.2 μm in diam; cystidia and cystidioles absent; basidia clavate, but narrower towards the base, hyaline, thin-walled, bearing a basal clamp connection and four sterigmata, 24–33 × 5–8 μm; basidioles in shape similar to basidia, but slightly smaller.

STIPE: Generative hyphae scanty; arboriform skeletal hyphae dominant, thick-walled with a narrow or wide lumen, moderately branched, aseptate, strongly interwoven, 1.5–7 μm in diam. Hyphae in black crust similar to those in stipe.

SPORES: Basidiospores cylindrical, hyaline, thin-walled, smooth, bearing a large guttule. IKI–, CB–, (7.8–)8–10(–10.5) × 3–3.9(–4) μm, L = 8.95 μm, W = 3.38 μm, Q = 2.63–2.67 (n = 60/2).

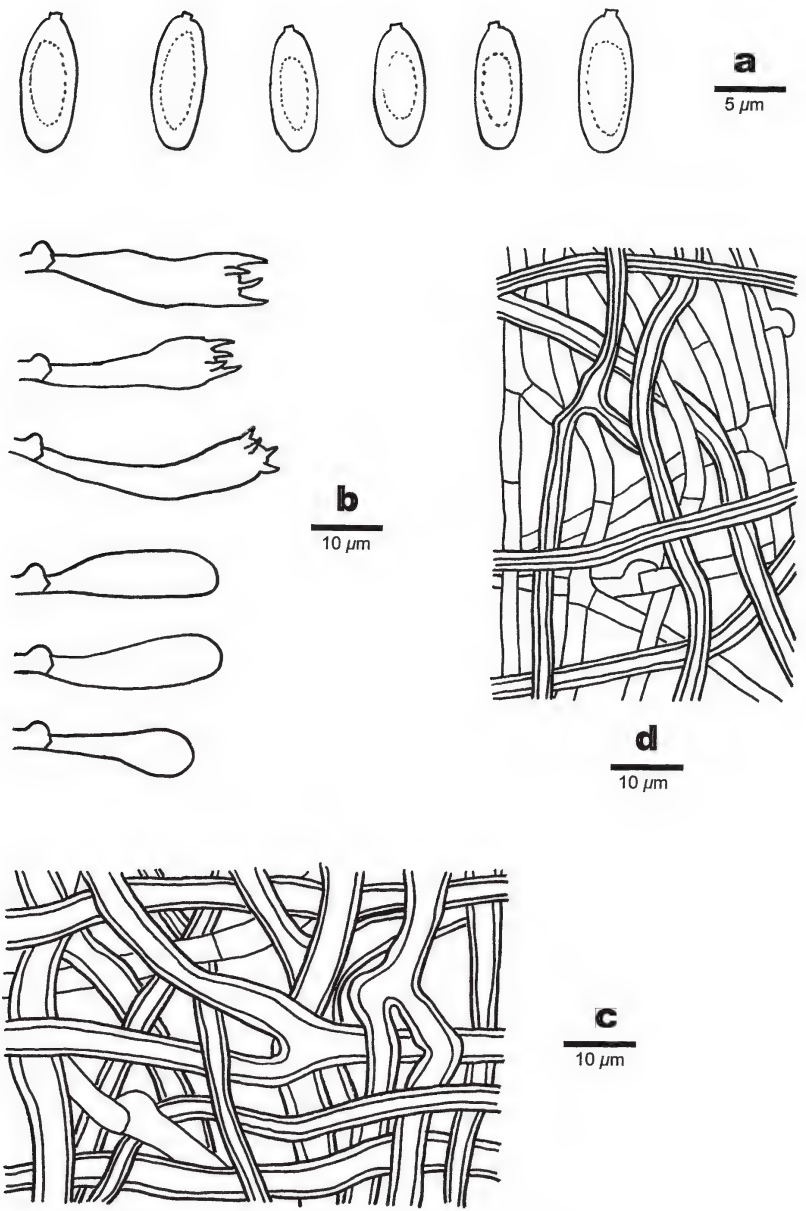


Fig. 1. *Polyporus submelanopus* (holotype).
a: Basidiospores. b: Basidia and basidioles.
c: Hyphae from context. d: Hyphae from trama.

Additional specimen examined: **CHINA**, **Qinghai Province**, Huzhu County, Beishan Forest Farm, on ground in forest of *Picea*, 1.IX.2003 Dai 4997 (IFP 004953; GenBank sequence JQ964424).

Remarks: *Polyporus submelanopus* is characterized by a terrestrial habit, centrally or laterally black-stipitate basidiocarps, straw-yellow to honey-yellow pore surface, circular to angular pores, two types of generative hyphae bearing either simple septa or clamp connections, and cylindrical basidiospores with a large guttule. Its black stipe and medium basidiospore size match the concept of the melanopus group (Núñez & Ryvarden 1995).

Polyporus melanopus resembles *P. submelanopus* in its similar pileus with a shallow central depression, circular to angular pore shape, basidiospores, and terrestrial habit. However, *P. melanopus* has slightly smaller pores (3–4 per mm, Gilbertson & Ryvarden 1987; 3–5 per mm, Bernicchia 2005; 3–4(–5) per mm, Boulet 2007), thicker and entire dissepiments, fusoid cystidioles, and generative hyphae bearing clamp connections only (Gilbertson & Ryvarden 1987).

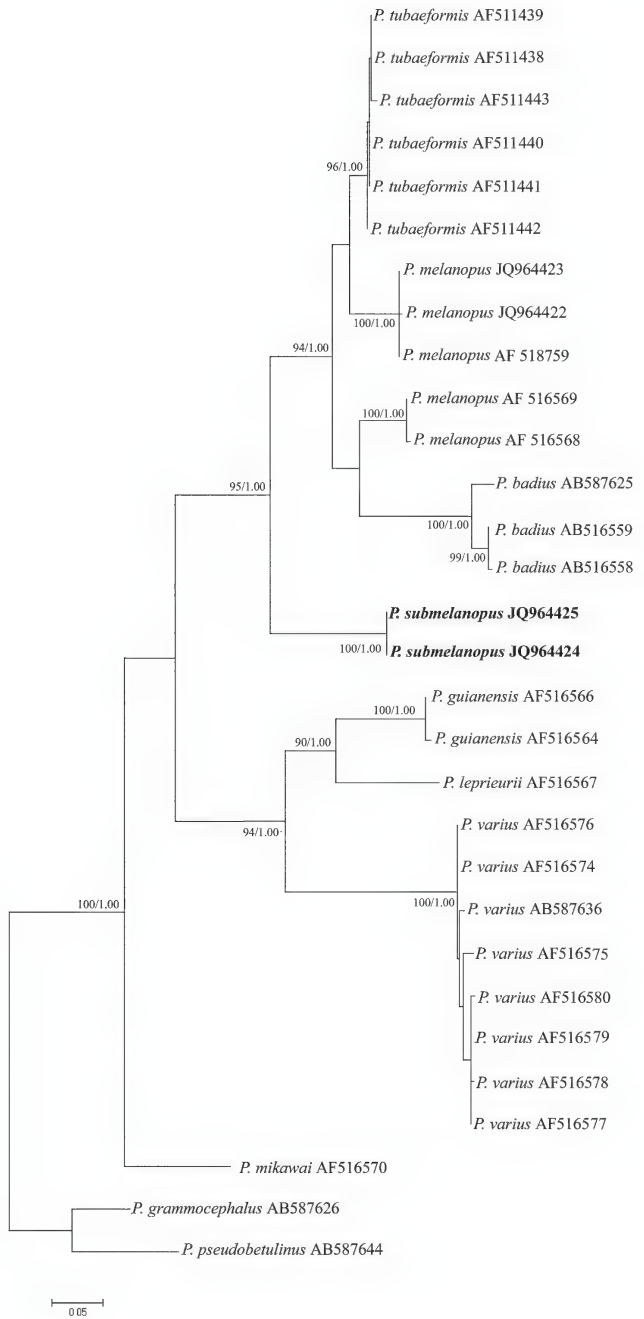
Polyporus badius (Pers.) Schwein., a species with simple-septate generative hyphae, is distinguished from *P. submelanopus* in darker pilei and smaller pores (5–8 per mm, Núñez & Ryvarden 1995). In addition, *P. badius* is a wood-inhabiting species rather than a terrestrial one.

Polyporus xinjiangensis J.D. Zhao & X.Q. Zhang, originally described from China, has generative hyphae bearing simple septa like *P. submelanopus* but produces no generative hyphae with clamp connections (Dai et al. 2007b); *P. xinjiangensis* further differs in its wood-inhabiting habit and slightly shorter basidiospores (7.1–8.5 µm long, Dai et al. 2007b).

Macroscopically, *P. submelanopus* shares similar infundibuliform pilei, a straw-yellow to pale brownish pore surface, and sharp margin with *P. tubaeformis* (P. Karst.) Ryvarden & Gilb., which is distinguished by possession of cystidioles and smaller pores [6–9(–10) per mm, (n = 150/5)] and basidiospores [(5.8–) 6–7.8(–8.2) × (2.1–)2.3–3.2(–3.5) µm, L = 6.49 µm, W = 2.75 µm, Q = 2.27–2.50 (n = 150/5), Dai 1996]. Besides, *P. tubaeformis* has only clamped generative hyphae and grows on wood rather than ground.

Our ITS dataset had 694 characters. Its best-fit evolutionary models for ML and Bayesian analyses were estimated as TrN + G and HKY + G, respectively. The phylogenetic tree (Fig. 2) formed of most members of melanopus group clusters *P. submelanopus* as a distinct clade within this group. Although we unfortunately failed to obtain an ITS sequence from *P. xinjiangensis,* the ecological habit and morphological differences above are enough to distinguish *P. submelanopus* from *P. xinjiangensis.*

Fig. 2. The phylogenetic tree inferred from ITS sequences of the members of Melanopus group. Topological structure was from ML analysis. The statistical values both above 75% of bootstrap value and 0.95 of Bayesian posterior probability are indicated.


P. tubaeformis AF511439
P. tubaeformis AF511438
P. tubaeformis AF511443
P. tubaeformis AF511440
96/1.00
P. tubaeformis AF511441
P. tubaeformis AF511442
P. melanopus JQ964423
P. melanopus JQ964422
100/1.00
P. melanopus AF 518759
94/1.00
P. melanopus AF 516569
100/1.00
P. melanopus AF 516568
95/1.00
P. badius AB587625
100/1.00
P. badius AB516559
99/1.00
P. badius AB516558
***P. submelanopus* JQ964425**
100/1.00
***P. submelanopus* JQ964424**
P. guianensis AF516566
100/1.00
P. guianensis AF516564
90/1.00
P. leprieurii AF516567
94/1.00
P. varius AF516576
P. varius AF516574
100/1.00
P. varius AB587636
100/1.00
P. varius AF516575
P. varius AF516580
P. varius AF516579
P. varius AF516578
P. varius AF516577
P. mikawai AF516570
P. grammocephalus AB587626
P. pseudobetulinus AB587644
0 05

The ITS tree also shows that the current concept of *P. melanopus* is not monophyletic. Five ITS sequences of *P. melanopus* formed two clades according to the specimens' origins. One clade comprised three European isolates, while two isolates from Argentina formed the other clade. Moreover, *P. melanopus* was described as a diverse species in pore and basidiospore size (Gilbertson & Ryvarden 1987, Bernicchia 2005, Niemelä 2005, Krüger et al. 2006, Boulet 2007). Therefore, more investigations are needed to clarify the exact circumscription of *P. melanopus*.

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A new species of *Clitopilus* from southwestern China

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Abstract — *Clitopilus ravus* is described and illustrated as a new species from southwestern China. It is characterized by its tawny gray or grayish brown to brown tomentose pileus, absence of pleuro- and cheilocystidia, subfusiform to amygdaliform basidiospores with 4–5 prominent longitudinal ridges, and cylindric or clavate terminal cells of the pileipellis filled with brown non-encrusting intracellular pigment. Comparisons with similar taxa and photographs and drawings of its macroscopical and microscopical characters are provided.

Key words — taxonomy, *Entolomataceae*, macrofungi

Introduction

The genus *Clitopilus* (Fr. ex Rabenh.) P. Kumm. traditionally encompasses three sections: *Clitopilus*, *Pleurotelloides*, and *Scyphoides*. Section *Clitopilus* is characterized by its medium-sized to rather large basidiomata, distinct and persistent stipe, spores with mostly six reinforced longitudinal angles and usually 10–14 µm long (Singer 1986). Seven species have been reported in sect. *Clitopilus*: *C. prunulus* (Scop.) P. Kumm., *C. lignyotus* Hongo, *C. quisquiliaris* (P. Karst.) Noordel., *C. paxilloides* Noordel., *C. griseobrunneus* T.J. Baroni & Halling, *C. amygdaliformis* Zhu L. Yang, and *C. chrischonensis* Musumeci et al. (Singer 1946; Hongo 1954; Noordeloos 1982, 1993; Baroni & Halling 2000; Yang 2007; Vizzini et al. 2011).

We describe and illustrate here a new species of sect. *Clitopilus* discovered during re-examination of *Clitopilus* specimens collected from Yunnan Province.

Materials & methods

Specimens were annotated and photographed in the field, and dried in an electric drier. Color description was according to Kornerup & Wanscher (1978). We examined

spores, basidia, and cystidia in 5% KOH or 1% Congo Red and the pileipellis in 5% KOH. Basidiospore dimensions follow the notation (a–)b–c(–d), with at least 25 basidiospores measured and with the range b–c representing 90% or more of the measured values and the extreme values 'a' and 'd' given in parentheses. Abbreviations are L_m = average length of all basidiospores ± sample standard deviation, W_m = average width of all basidiospores ± sample standard deviation, Q = individual basidiospore length/width ratio, Q_m = average Q of all basidiospores ± sample standard deviation, and n = number of spores measured per specimen.

Micrographs were taken using an Olympus BX51 trinocular phase contrast microscope and a Philips FEI-XL30 scanning electron microscope. The dried studied specimens are deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (KUN, with HKAS numbers).

Taxonomy

Clitopilus ravus W.Q. Deng & T.H. Li, **sp. nov.** Fig. 1

MycoBank MB 800634

Differs from *C. griseobrunneus* by its much smaller basidiospores and non-encrusting pigment in pileipellis.

Type: China, Yunnan Province, Jingdong County, Ailaoshan National Nature Reserve, 14 July 2008, Yanchun Li 1213 (**Holotype**, HKAS 56067).

Etymology: *ravus* refers to the tawny gray or grayish brown color of pileus and stipe.

Pileus 30–40 mm broad, convex at first, then applanate, tawny gray or grayish brown to brown (6D3, 6D–E5); brown (6E5) overall under lens, tomentose; margin incurved, not striate. Lamellae white but soon pale flesh-pink to pink (7A1–2), decurrent, close to crowded; edges concolorous and even; lamellulae numerous. Stipe paler than pileus to partially concolorous with pileus, orange gray (6B2), brownish orange (6C3) to light brown (6D4), with white (6A1) cottony mycelium at the base, 35–45 mm long, 4–6 mm thick at the apex, 7–12 mm thick at the base, tomentose, inconspicuously striate, central to slightly eccentric, often slightly curved, cylindrical, usually broadening towards base. Context white (6A1), unchanging when bruised or exposed to air.

Basidiospores 11–13(–14) × (5–)5.5–6.5(–7) μm, (n = 25, L_m = 12.02/± /0.96, W_m = 6.06/± 0.46, Q = (1.57–)1.83–2.33(–2.80), Q_m = 1.99 ± 0.25), slightly pinkish to nearly colorless and hyaline, slightly thick-walled, amygdaliform or subfusiform with longitudinal ridges in profile and face views, angular in polar view with 4–5 facets separated by obvious rounded ridges. Basidia (22–)27–35 × 10–13 μm, clavate, hyaline, 4-spored; sterigmata 2.5–3.0 μm long. Subhymenium composed of hyphal elements 5–9 μm in diam. Lamellar trama composed of cylindric, hyaline hyphae 2–6 μm in diam. Pleurocystidia and Cheilocystidia absent. Pileipellis a trichoderm composed of repent, cylindric or inflated, 5–11 μm wide hyphae; terminal cells cylindric or clavate,

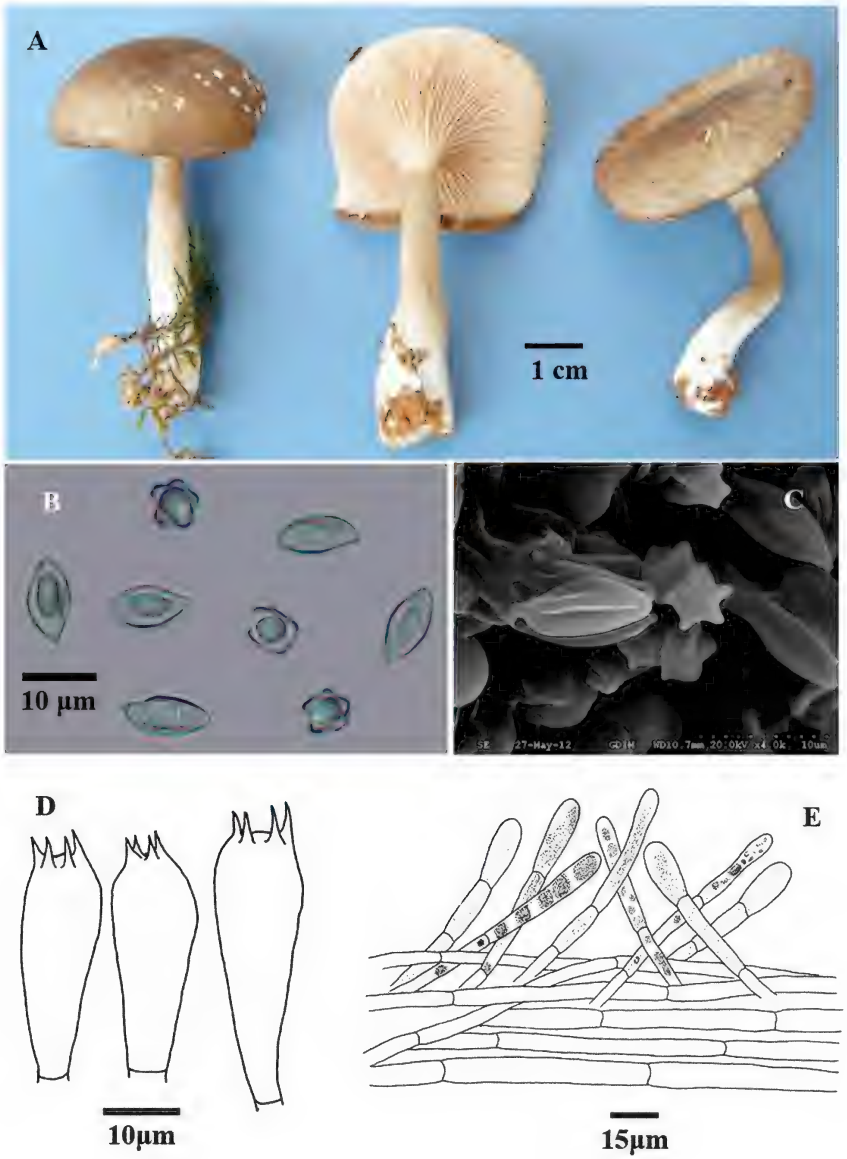

Fig. 1: *Clitopilus ravus* (holotype).
A. Basidiomata.
B. Basidiospores in LM (×1000). C. Basidiospores under SEM. D. Basidia. E. Pileipellis.

15–38 × 5–11 μm; brown, intracellular, non-encrusting pigments, some hyphae have thickened walls. Clamp connections absent in all tissues.

Ecology & distribution — Solitary or scattered on soil under broadleaf trees.

Comments — *Clitopilus ravus* is characterized by its tawny gray or grayish brown to brown tomentose pileus, absence of pleuro- and cheilocystidia, subfusiform to amygdaliform basidiospores with 4–5 prominent longitudinal ridges, and pileipellis cylindric or clavate terminal pileipellis cells filled with brown non-encrusting intracellular pigment. We place the new species in *Clitopilus* sect. *Clitopilus* based on its well-developed stipe and large basidiospores with fewer than 7 prominent longitudinal ridges (Singer 1986).

Basidiomes are easily distinguished from the white or pale grayish basidiomata of *C. prunulus*, *C. amygdaliformis* and *C. chrischonensis*. With *C. lignyotus*, *C. quisquiliaris*, *C. paxilloides,* and *C. griseobrunneus,* the new species represents the fifth gray or brown member of sect. *Clitopilus*. Although somewhat similar to *C. ravus* in color and stature, *C. lignyotus* is distinguished by a clitocybe-like or (often) phylloporus-like appearance and slightly smaller basidiospores (9.5–13 × 4.5–6.0 μm) with 6 longitudinal ridges (Hongo 1954). *Clitopilus quisquiliaris* differs distinctly in its reddish brown or fuscous brown pileus and smaller basidiospores (8–9.5 × 4.0–5.0 μm) (Noordeloos 1982; Hansen & Knudsen 1992); *C. paxilloides* has a weakly hygrophanous gray-brown pileus with numerous gray-brown spots, a strongly tapering stipe base, and obviously encrusted hyphae in the pileipellis (Noordeloos 1993); and *C. griseobrunneus* has much larger basidiospores (9.7–16.9 × 5.7–7.9 μm) with (5–) 6 (–7) longitudinal ridges and encrusted hyphae in the pileipellis (Baroni & Halling 2000).

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Arthrinium rasikravindrii sp. nov. from Svalbard, Norway

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Abstract — *Arthrinium rasikravindrii* sp. nov. was isolated from soil collected in the arctic archipelago Svalbard, Norway. The new species, distinguished by morphological and in vitro cultural characters, forms dark brown lenticular conidia with hyaline equatorial germ slits together with balloon-shaped, anomalous conidia uncommon in the morphologically similar *A. phaeospermum*. Additionally, internal transcribed spacer (ITS) rDNA sequence analyses support this species as distinct within *Arthrinium*. Isolates previously identified as *A. phaeospermum* from China and Japan are re-determined as *A. rasikravindrii*.

Key words — anamorphic fungi, Ny-Ålesund, phylogeny, taxonomy

Introduction

The study of fungal diversity in soils from Svalbard, Norway (Elvebakk et al. 1996, Aarnæs 2002, Kurek et al. 2007), has led to the discovery of novel genera and species from this region (Pang et al. 2008, 2009). During research of the soil mycobiota of this Arctic region, a previously unknown *Arthrinium* was isolated from a soil sample. *Arthrinium* Kunze was established in 1817 with *A. caricicola* Kunze as the type species (Kunze 1817). The genus currently comprises about 31 species worldwide (Kirk et al. 2008), of which 13 have been reported from Svalbard (Aarnæs 2002). Most *Arthrinium* species produce dark, non-septate, mostly lenticular conidia with a hyaline rim or germ slit. Since Ellis (1965, 1971, 1976) extensively treated the genus, several species have been added (Calvo & Guarro 1980, Larrondo & Calvo 1990, 1992). This paper describes and illustrates another new *Arthrinium* species isolated from arctic soil near Svalbard, Norway.

Materials & methods

Sampling site

The soil sample was collected from Ny-Ålesund (78°55′N 11°56′E) on the west coast of Spitsbergen, the largest island in the Svalbard archipelago. Topographically, Ny-Ålesund includes east and west glaciers, terminal moraines, and glacial streams and rivers flowing towards Kongsfjord. The sampling site was situated near an Austre Brøggerbeen glacier (78°55.082′N 11°51.527′E). Mean temperatures are –14°C in the coldest month (February) and 5°C in the warmest month (July–August) (Nygaard 2009). The loose soil texture supports a tundra vegetation (Klimowicz & Uziak 1998).

Isolation, pure culture, and microscopic examination

The soil was sampled from the surface to 5 cm depth from the Ny-Ålesund region during the first (2007) Indian Arctic expedition. The samples were placed in sterile ampoules (Hi-media) and stored at –20°C until studied. For the isolation of fungi, 1 gm of soil sample was taken and serially diluted up to 10^{-7} (Waksman 1916). A soil suspension (1 ml) was used as inoculum on four different culture media — Martin's Rose Bengal medium (MRB), Malt Extract Agar (MEA), Corn Meal Agar (CMA), and Potato Dextrose Agar (PDA). The plates were incubated at 15°C for 10 days. Colonies showing different morphological features were purified and transferred onto PDA slants for further study. Sporulating cultures were identified based on morphology using standard literature (Ellis 1971, 1976, Carmichael et al. 1980, Domsch et al. 1980, Larrondo & Calvo 1990, 1992). We recorded and photographed microscopic details from specimens mounted in lactophenol-cotton blue and distilled water using an Olympus CX-41 microscope. Fungal structures were measured with an ocular micrometer. A pure culture is deposited in the National Fungal Culture Collection of India (NFCCI-WDCM 932), MACS' Agharkar Research Institute, Pune, India.

Polymerase chain reaction (PCR) and sequencing

Total DNA was extracted from a culture grown on a PDA plate for two weeks at 15°C by the high salt DNA extraction method of Aljanbi & Martinez (1997) using Fast Prep-24 tissue homogenizer (MP Biomedicals GmbH, Germany). ITS fragments were amplified using primer pairs ITS4 and ITS5 (White et al. 1990). PCR was performed in a 25 µl reaction using 2 µl template DNA (10–20 ng), 0.5 U Taq DNA polymerase (Genei, Bangalore, India), 2.5 µl 10X Taq DNA polymerase buffer, 0.5 µl 200 µM of each dNTP (Genei, Bangalore, India), 0.5 µl 10 pmol primer, H_2O (Sterile Ultra Pure Water, Sigma) qsp 25 µl. Amplification was performed on an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) using the following parameters: 5 min step at 95°C, followed by 30 cycles of 1 min at 95°C, 30s at 56°C, and 1 min at 72°C for ITS region amplification, then a final 7 min extension step at 72°C. The PCR products were purified with Axygen PCR cleanup kit (Axygen Scientific Inc, CA, USA) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The cycle sequencing products were run on an ABI Avant 3100 automated DNA sequencer (Applied Biosystems, USA).

Sequence alignment and phylogenetic analysis

Sequence alignment of internal transcribed spacer and 5.8S regions of *Arthrinium* sp. (NFCCI 2144) was performed using the Clustal W option of the software Molecular

Table 1. Percent ITS sequence similarity of *Arthrinium rasikravindrii* with Genbank sequences from related *Arthrinium* species.

Species	Culture #	Genbank #	Positions with base changes	Total aligned sequence bp	Sim. w nfcci2144 (%)*
A. rasikravindrii	nfcci 2144	JF326454	-	-	-
#	ifo 6575	ab220266	1	501	99.8
#	maff 305708	ab220272	1	501	99.8
#	dlen2008007	gu266274.1	1	501	99.8
#	l 10-2-2	hm008625.1	3	501	99.4
#	l 3-4-2	hm008624.1	2	501	99.6
#	maff 410785	ab220273.1	3	501	99.4
A. aureum Calvo & Guarro	cbs 244.83	ab220251	12	502	97.6
	imi 252326d	ab220246	12	502	97.6
A. arundinis (Corda) Dyko & B. Sutton	imi 285638b	gu566268	86	521	83.5
A. euphorbiae M.B. Ellis	g41	gu566268	67	503	86.7
A. hispanicum Larrondo & Calvo	imi 326877	ab220242	64	521	87.7
A. marii Larrondo & Calvo	cbs 497.90	ab220252	65	522	87.5
A. mediterranei Larrondo & Calvo	imi 326875	ab220243	62	521	88.0
A. phaeospermum (Corda) M.B. Ellis	maff 236534	ab220270.1	34	347	90.2
	maff 235494	ab220269.1	34	346	90.2
	cbs 463.83	aj279447	50	503	90.1
	cbs 142.55	ab220256	51	503	89.8
	xsd-91	eu326184.1	35	345	89.8
	xsd-132	eu326200.1	35	345	89.8
	a229	hm222956.1	35	345	89.8
	xsd-08044	fj478101.1	36	347	89.6
	maff 236535	ab220271.1	36	347	89.6
	mucl 8362	ab220283.1	36	347	89.6
	oucmbi091005	hq914944.1	36	347	89.6
	oucmbi101209	hq914942.1	36	347	89.6
	ifo 31950	ab220267.1	36	346	89.6
	a218	hm222950.1	36	346	89.6
	a3	aj279456.1	37	346	89.3
	dsm 62039	ab220261.1	37	345	89.2
A. phaeospermum ?	t57	fj462766.1	65	521	87.7
A. phaeospermum ?	63/2.4	dq865110	53	398	86.7
A. puccinioides Kunze	cbs 549.88	ab220253.1	83	347	76.0
A. serenense Larrondo & Calvo	cbs 498.90	ab220240	52	503	89.6
A. sacchari (Speg.) M.B. Ellis	cbs 334.86	ab220257	52	503	89.6
A. saccharicola F. Stevens	atcc 76288	ab220238	52	503	89.6
Apiospora montagnei Sacc.	cbs 301.49	ab220258	66	521	87.3
Nigrospora sphaerica (Sacc.) E.W. Mason	9038	gq919077			

* ITS1-5.8S-ITS2 sequence of NFCCI 2144 was subjected to pairwise alignment using Clustal W.

Designated as *Arthrinium phaeospermum* in Genbank

Evolutionary Genetics Analysis (MEGA) software v5.0. (Tamura et al. 2011). The manually edited sequence of NFCCI 2144 was deposited in the NCBI nucleotide sequence database (JF326454) and subjected to a NCBI BLAST search. The partial ITS sequences were aligned using Clustal W together with homologous ITS sequences retrieved from Genbank of the same and related species of *Arthrinium* (TABLE 1). Due to inconsistent sequence lengths, portions of the ITS1 and ITS2 were excluded from the analysis. To calculate the sequence divergence for ITS, the matrix was analyzed with the Neighbour Joining method (Saitou & Nei 1987) using the Kimura-2 parameter model (Kimura 1980) and Maximum Parsimony method (Tamura et al. 2011). The bootstrap consensus tree (Felsenstein 1985) was inferred from 1000 replicates, with all gaps and missing data eliminated from the dataset (Complete Deletion option).

Taxonomy

Arthrinium rasikravindrii Shiv M. Singh, L.S. Yadav, P.N. Singh, Rahul Sharma & S.K. Singh, **sp. nov.** FIGS 1, 2I–P

MYCOBANK MB 800216

Differs from *Arthrinium phaeospermum* by its larger lenticular conidia and its production of a second elongate type of conidia.

TYPE: Norway, Svalbard, Spitsbergen, NyÅlesund, 78°55'N 11°56' E, from soil, 27.07.2010, leg. S.M.Singh, (**Holotype,** AMH 9435 [dried colony on PDA]; ex-type culture, NFCCI 2144; GenBank JF326454).

ETYMOLOGY: The specific epithet refers to Dr Rasik Ravindra, director of the National Centre for Antarctic & Ocean Research and leader of the first Indian Arctic Expedition.

Conidiophores arising from swollen basal cells, micro-semi-macronematous, mononematous, straight or flexuous, unbranched, septate, smooth, thin-walled, hyaline to sub-hyaline, 5–90 × 1–1.5 µm. Conidia arising acropleurogenously, dimorphous i) lenticular, ovoid in face view, 10–15 × 6.0–10.5 µm (n = 50); ii) elongate to clavate conidia, 15–25 × 7.5–10 µm (n = 50), smooth, double-walled, brown to pale olivaceous with prominent truncate base and equatorial germ slit.

Optimum temperature for growth 15–18°C for the arctic holotype (but 25°C for isolates from China and Japan). Colonies on PDA, medium to fast growing, 45–70 mm in 7 days at 18°C (arctic isolate) or 25°C with white, floccose aerial mycelium, reverse uncolored, yellow bright to buff (Rayner 1970). Colony of MAFF410785 slow growing 17.0 mm in 7 days at 25°C, raised dense cottony unusually different from other strains. Colony pattern differing among all four strains tested. Individual strains on OA, PCA, and MEA with similar morphology as on PDA. Sporulation observed after 7 days to 45 days on PCA, as dark pinhead spots in the aerial mycelium near the periphery and later the centre of the colony.

TELEOMORPH: not observed.

DISTRIBUTION—China, Japan, Norway.

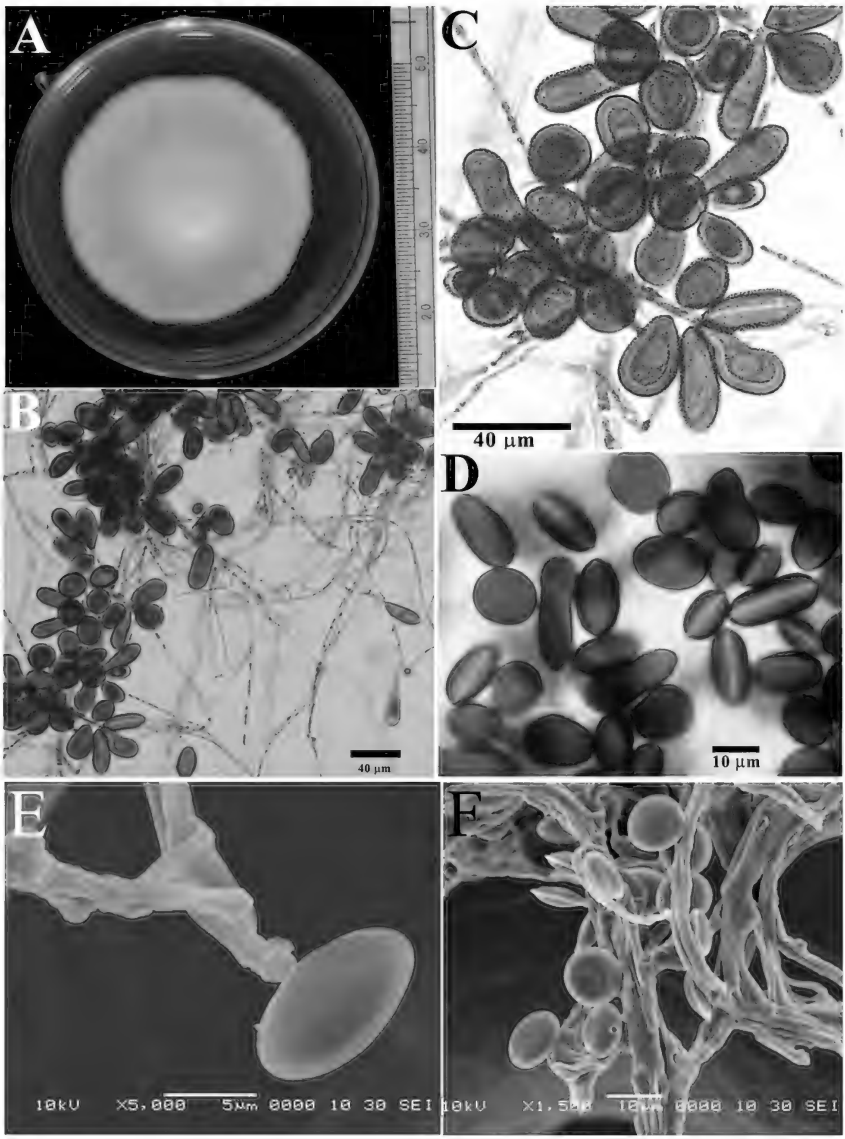


FIG. 1. *Arthrinium rasikravindrii* (NFCCI 2144[T]). A: 7-day old colony on PDA. B–C: Conidia attached to conidiophores. D: Enlarged anomalous and lenticular conidia. E–F: Conidium and conidia under SEM.

ADDITIONAL MATERIAL EXAMINED—**JAPAN**: Fukushima, isolated from leaf of *Coffea arabica,* T. Sato (MAFF305708); Chiba, isolated from wood tissue of *Pinus thunbergii,* T. Kobayasi (MAFF410785); T. Hasegawa (NBRC6575, IFO6575).

Additional sequenced isolates—**CHINA**: Dalian, isolated from submerged wood collected from marine coast (DLEN2008007); isolated as endophytes from *Oryza granulata* (L10-2-2, L3-4-2). **JAPAN**: Hiroshima, isolated from stubble of *Triticum aestivum*, T. Aoki (MAFF236535, an authentic *A. phaeospermum* strain).

Discussion

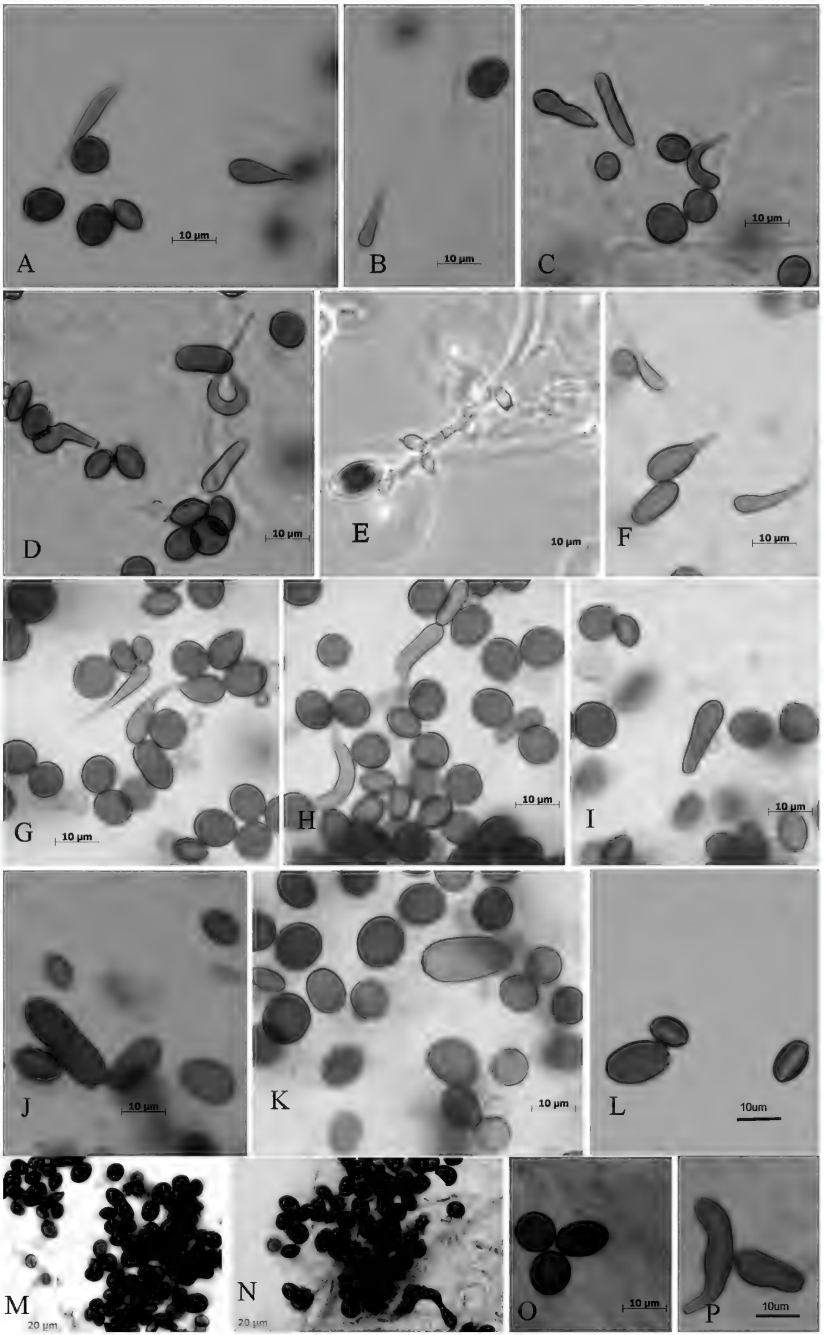
Based on morphological characters and ITS sequence similarity (NCBI BLAST search), the Arctic isolate was determined to belong to *Arthrinium*. Most *Arthrinium* species produce lenticular conidia. As the new species abundantly produces conidia that are lenticular but also elongate, it differs from any previously described *Arthrinium* species, which rarely or never produce elongate conidia.

The NFCCI2144 ITS region comprised a 534 bp nucleotide sequence with 222 ITS1 bp, 150 5.8S bp, and 160 ITS2 bp. A BLASTn search against the Genbank DNA database with this ITS sequence showed maximum similarity (99%) with ambiguous *A. phaeospermum* strains (HM008624.1 query coverage 93%; HM008625.1 query coverage 94%), 97% similarity with *A. aureum* (AB220246.1 query coverage 88%; AB220251.1 query coverage 87%), and only 90% similarity with the isotype strain from *A. phaeospermum* (AB220283.1 query coverage 88%).

A previous study by Khan et al. (2009) of *A. phaeospermum* involving 16 Genbank sequences showed considerable variability among the deposited sequences. Our search of Genbank for *A. phaeospermum* ITS sequences as revealed 25 accessions. A Neighbor Joining tree constructed using these 24 sequences + NFCCI2144 revealed three groups. Our Arctic isolate clustered with six *A. phaeospermum* sequences including IFO6575, MAFF410784, and MAFF305708, which are not monophyletic with the ex-isotype strain of *A. phaeospermum* CBS142.55. Examination of strain IFO6575 revealed the anomalous conidia characteristic of *A. rasikravindrii* and measuring 9.5–22.5 µm × (6.5–)8.5–14.5(–10) µm. Morphological examination of MAFF410785 and MAFF305708 (Fig. 2I–L, O, P) belonging to clade Ia (Fig. 3) and clade IIb (Fig. 4) also showed conidial morphology typical of *A. rasikravindrii*. Their ITS sequences showed a 99.4% and 99.8 % similarity, respectively, to the *A. rasikravindrii* ex-type strain.

Phylogenetic analysis of 10 related species of *Arthrinium* using neighbor-joining method (Fig. 3) placed *Arthrinium* spp. into three clades: clade I contains two subclades — Ia with *A. rasikravindrii* and Ib with two *A. aureum* strains —

Fig. 2. **A–H.** *Arthrinium phaeospermum* (MAFF 236535). A–D, F–H: variously shaped conidia—typical lenticular, globose, and narrow elongated. E: conidiophores. **I–P.** *Arthrinium rasikravindrii*. I–L (MAFF 305708): lenticular, globose, and anomalous conidia. M–N (IFO 6575): lenticular, globose, and anomalous conidia. O–P (MAFF 410784): globose and anomalous conidia.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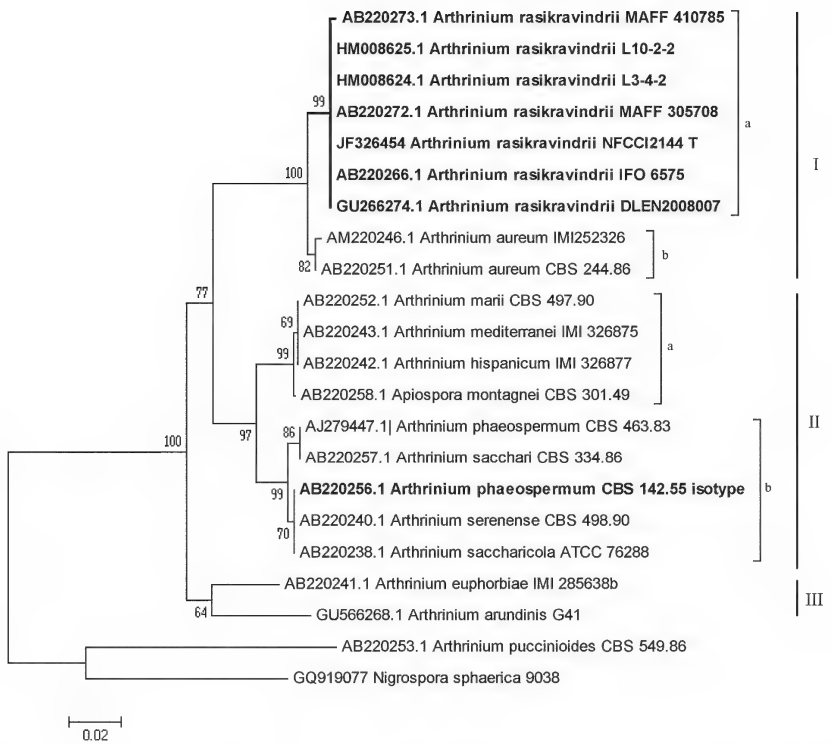

FIG. 3. Neighbor joining tree (1000 bootstrap replications) showing phylogenetic relationship of *Arthrinium rasikravindrii* NFCCI 2144 ^{T} and 22 ITS sequences deposited in Genbank of authentic strains of 13 *Arthrinium* species. The tree scale (0.02) represents evolutionary distances in units of base substitutions per site as computed by Kimura-2 parameter method. Bootstrap values more than 50 are shown. Gaps are treated as missing data and eliminated from dataset during tree construction. *Nigrospora sphaerica* (GQ919077.1) was taken as outgroup.

clade II includes the most species (8), and clade III comprises *A. euphorbiae* and an *Arthrinium* strain wrongly identified as *A. arundinis* (*Apiospora montagnei*) that is non-monophyletic with the authentic strain of *Apiospora montagnei* CBS 301.49 (clade IIa). Strain IFO6575 and five others are considered to represent *A. rasikravindrii* rather than *A. phaeospermum* as listed in Genbank because they cluster with the holotype isolate and are distant from the isotype strain CBS142.55 of *A. phaeospermum* (TABLE 1).

A combined analysis of *Arthrinium* spp. (FIG. 4) including all *A. phaeospermum* accessions was consistent with FIG. 3 except that *A. phaeospermum* strains were distributed in three major clusters suggesting fungal sequences incorrectly

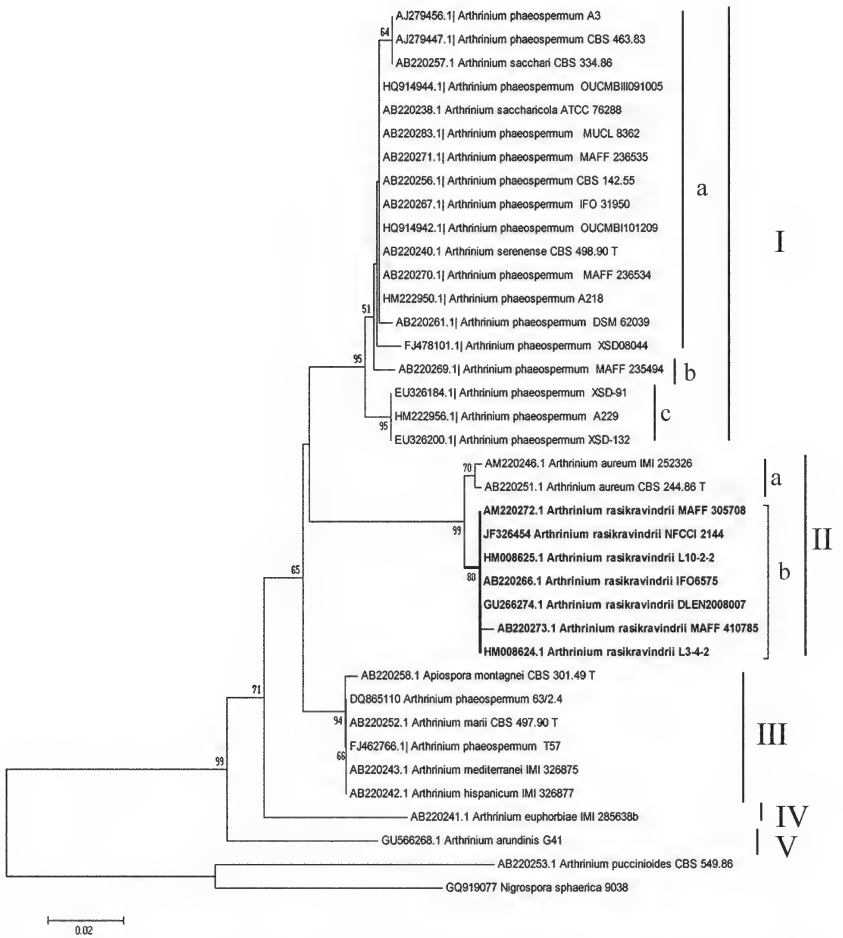

FIG. 4. Neighbor joining phylogenetic tree (1000 bootstrap replications) constructed using ITS sequences of NFCCI 2144^{T} and 24 accessions deposited in Genbank as *Arthrinium phaeospermum* plus accessions of 13 *Arthrinium* spp. The tree scale (0.02) represents evolutionary distances in units of base substitutions per site as computed by Kimura-2 parameter method. Bootstrap values more than 50 are shown. Gaps are treated as missing data and eliminated from dataset during tree construction.

assigned to *A. phaeospermum*. Two strains designated as *A. phaeospermum* belonging to clade III in FIG 4 may also have been misidentified because they are not monophyletic with the *A. phaeospermum* ex-isotype culture CBS142.55 but group instead with *A. hispanicum*, *A. marii*, and *A. mediterranei*. Also three '*A. phaeospermum*' in FIG 4 clade Ic represent an undescribed species.

Arthrinium rasikravindrii differs morphologically from *A. phaeospermum* in having larger conidia and producing a second type of conidia, also seen in an unidentified species of *Arthrinium* by Adelantado et al. (2010) and the *Arthrinium* state of *Apiospora montagnei* by Minter (1985). Additionally *A. phaeospermum* forms narrowly elongated pale brown conidia (Fig. 2A–D, F–H) not seen in any of the examined *A. rasikravindrii* strains (Figs 1, 2I–P). Although *A. phaeospermum* and the *Arthrinium* state of *Apiospora montagnei* form anomalous conidia like *A. rasikravindrii,* these occur only rarely. Genetically, *A. rasikravindrii* differs in 51 positions (10.2%) from *A. phaeospermum* CBS142.55 out of the 503 nucleotides in the ITS region. *Arthrinium rasikravindrii* differs from *A. aureum* having conidia 14–30 × 10–15 µm, and these species differ in the ITS region at 12 nucleotide positions (2.4%). All other species are more than 10% distant from *A. rasikravindrii* in the ITS region. None of the known *Arthrinium* species of form elongate truncate conidia (referred to as anomalous conidia by Adelantado et al. 2010) in abundance except *A. rasikravindrii* (Fig. 1B–D) and the *Arthrinium* state of *Apiospora montagnei* that is genetically (>10%) distant from *A. rasikravindrii* and *A. phaeospermum*.

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New records of *Ochrolechia* and *Placopsis* from the Hengduan Mountains, China

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Abstract—Based on specimens collected in the Hengduan Mountains, *Ochrolechia laevigata* is recorded as new to Asia, *O. africana* as new to China, and *Placopsis gelida* as new to mainland China.

Key words — *Ochrolechiaceae*, *Trapeliaceae*, taxonomy, Yunnan

Introduction

Ochrolechia A. Massal. (*Ochrolechiaceae*, *Pertusariales*, *Ascomycota*), a widespread genus of crustose lichens, comprises about 60 species worldwide (Verseghy 1962; Howard 1970; Brodo 1987, 1991; Lumbsch et al. 2003; Kirk et al. 2008; Kukwa 2009, 2011). In China, 25 species have been reported (Wei 1991, Jia & Zhao 2003, Jia et al. 2008), of which 18 are known from Southwest China. During our study of lichens collected from the Hengduan Mountains, we recorded *O. africana* for the first time from China and *O. laevigata* for the first time from Asia. We also collected in the same area *Placopsis gelida,* reported here as new to mainland China.

Materials & methods

The specimens studied are preserved in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University). The morphological and anatomical characters of the specimens were examined under a stereomicroscope (Olympus SZ) and a polarizing microscope (Olympus CX21). The lichen substances were identified using the standardized thin layer chromatography techniques (TLC) with C system (Orange et al. 2010) and mercury cadmium telluride [MCT] crystal analysis. Photographs were taken of the morphology with an Olympus SZX16 stereomicroscope and of the anatomy with an Olympus BX61 compound microscope with DP72.

New records

Ochrolechia africana Vain., Ann. Univ. Fenn. Aboënsis, ser. A, 2(3): 3. 1926. FIG. 1

MORPHOLOGY — THALLUS crustose, yellowish grey to grey, thin to thick and verruculose to verrucose; ISIDIA AND SOREDIA absent; APOTHECIA sessile, numerous, 1.0–1.8 mm in diam.; DISK light yellowish pink to light orange, with white and scabrose pruina; THALLINE MARGIN usually concolorous with the thallus, margins thick, dull, smooth, often prominent; EPITHECIUM pale brown; HYMENIUM 220–330 μm high; HYPOTHECIUM 35–40 μm high; EXCIPULUM PROPRIUM 15–25 μm; AMPHITHECIUM with a thick, well-developed medulla, sometimes with small crystals, and thin cortex (30 μm thick); ALGAL LAYER virtually continuous below hymenium or sometimes sparse; ASCI 8-spored; ASCOSPORES simple, ellipsoid, 55–62.5 × 30–32.5 μm; PYCNIDIA absent.

SPOT TESTS — Thallus cortex K–, C–, KC–; medulla K–, C+ red, KC+ red; apothecial disk K+ yellow, C+ red, KC+ red; apothecial margin cortex K–, C–, KC–; apothecial margin medulla C+ red; thallus UV–.

SECONDARY METABOLITES — Gyrophoric acid with traces of lecanoric and 4-*O*-methylhiascic acids.

DISTRIBUTION & HABITAT — *Ochrolechia africana* is a pantropical species, reported from southeastern coastal plain, tropical and subtropical areas in

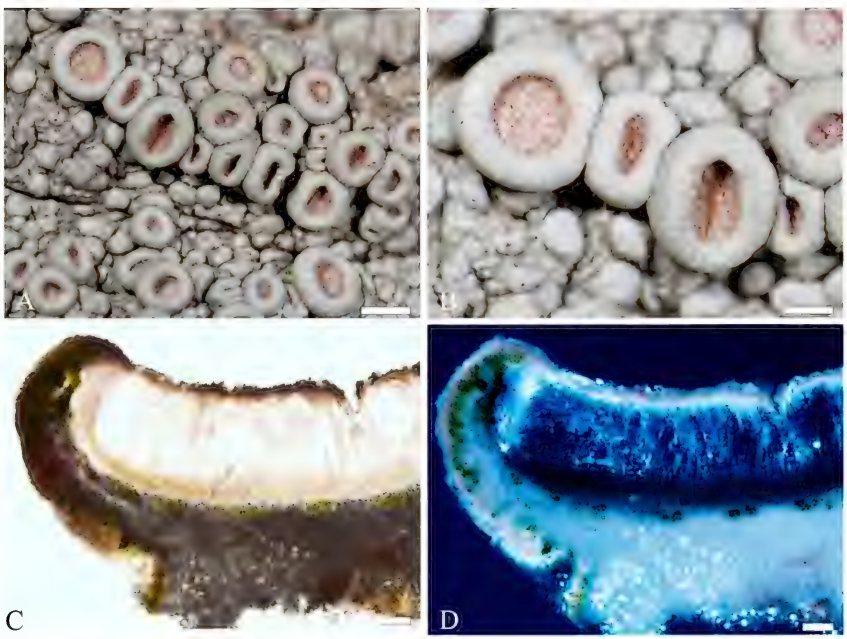

FIGURE 1. *Ochrolechia africana* [Z.J. Ren 20113910]. A. Thallus. B. Apothecia. C. Apothecial section. D. Crystals in apothecial section. Scale bars: A = 1 mm; B = 0.3 mm; C, D = 100 μm.

North and South America, Asia, Australia, and southern Africa (Awasthi & Tewari 1987, Brodo 1991, Aptroot & Feijen 2002, Roemer et al. 2004). The species grows on wood and the bark of deciduous trees.

Specimen examined — **CHINA. Yunnan.** Lijiang county, Mt. Laojun, alt. 2800 m, on bark, 5 Nov. 2009, Z.J. Ren 20113910 (SDNU).

Comments — *Ochrolechia africana* is characterized by small, pruinose apothecia with smooth apothecial margins, a C+ red medulla due to the production of hiascic acids, and a C– negative amphithecial cortex. The species may be confused with *O. mexicana*, which can be distinguished by a C+ red apothecial cortex, a usually more pruinose disk, and the absence of an excipular ring around the disk (Brodo 1991).

Ochrolechia laevigata (Räsänen) Verseghy ex Kukwa, Lichen Genus *Ochrolechia* in Europe: 132. 2011. Fig. 2

Morphology — Thallus crustose, yellow to pale yellow, shiny, very thin, continuous, thin to moderately thick, not verrucose; apothecia circular, shallow saucer-shaped, usually regular in outline, 1.0–2.4 mm in diam., sessile, light orange, epruinose; thalline margin 0.2–0.6 mm thick, entire, smooth, always concolorous with the thallus; epithecium faintly brownish; hymenium 300–340 µm high; hypothecium about 90 µm high, gray; amphithecium medulla lax, alga layer almost absent, or sometimes only in upper lateral margins not below hypothecium; cortex 45 µm thick laterally, expanding to 100 µm at base; excipulum proprium distinct below hypothecium, 50–60 µm thick; asci 6–8 spored; ascospores simple, ellipsoid, 70–75 × 32.5–40 µm; pycnidia absent.

Spot tests — Thallus cortex K+ yellow, C+ red, KC+ red; medulla K–, C+ red, KC+ red; apothecial disk K–, C+ red, KC+ red; apothecial margin cortex K± yellow, C+ red, KC+ red; margin medulla C+ faintly red.

Secondary metabolites — Gyrophoric acid with a trace of lecanoric acid.

Distribution & habitat — *Ochrolechia laevigata,* which has been previously reported only from the west coast of North America (Brodo 1991, Brodo et al. 2003), grows on deciduous trees.

Specimens examined — **CHINA. Yunnan.** Lijiang county, Mt. Laojun, alt. 4000 m, on bark, 5 Nov. 2009, Q. Tian 20100498, 20100502 (SDNU).

Comments —*Ochrolechia laevigata,* which is characterized by a thin thallus, apothecial margins almost without an algal layer, and a distinct excipulum proprium, resembles *O. oregonensis* and *O. subpallescens* morphologically, but *O. oregonensis* has a thick, verruculose to verrucose thallus while *O. subpallescens* has a continuous algal layer under the hymenium (Brodo 1991). Although our Chinese specimen does not contain olivetoric and 4-*O*-demethylmicrophyllinic

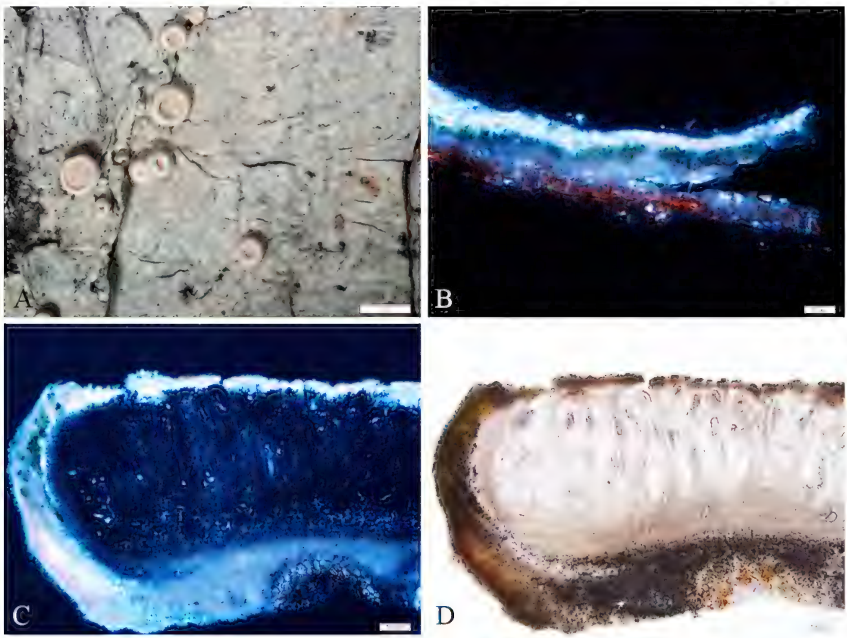

FIGURE 2. *Ochrolechia laevigata* [Q. Tian 20100498]. A. Thallus. B. Crystals in thallus section. C. Algal layer section. D. Excipulum proprium below hypothecium. Scale bars: A = 2 mm; B = 50 μm; C, D = 100 μm.

acids, it agrees morphologically with the description given by Brodo (1991). The chemical variation of the species may be greater than previously reported and should be studied further.

Placopsis gelida (L.) Linds., Trans. Linn. Soc. London 25: 536. 1866. FIG. 3

MORPHOLOGY — THALLUS dull grey, cream, pinkish or pale brown, forming rosettes; LARGE CENTRAL with cracked to rimose-areolate part, with radiating marginal lobes, 0.4–1.3 mm wide; SURFACE with pinkish to grey-brown, epruinose, CEPHALODIA deeply plicate, 0.4–4 mm in diam.; SORALIA farinose green-grey to grayish, generally delimited by a sharply defined and slightly raised margin; APOTHECIA 0.7–1.8 mm in diam., sessile, pinkish to yellow-brown, often with whitish pruinose; ASCI 8-spored, cylindrical, thin walled; ASCOSPORES colourless, simple, ellipsoid, 15–17.5 × 8–10 μm.

SPOT TESTS — Thallus cortex K–, C+ red, KC+ red; medulla K–, C+ red, KC+ red; apothecial disk K–, C+ red, KC+ red; apothecial margin cortex K–, C+ red, KC+ red.

SECONDARY METABOLITES — Contains only gyrophoric acid.

DISTRIBUTION & HABITAT — *Placopsis gelida* is a widespread, saxicolous, oceanic to arctic species, reported from both the northern and southern

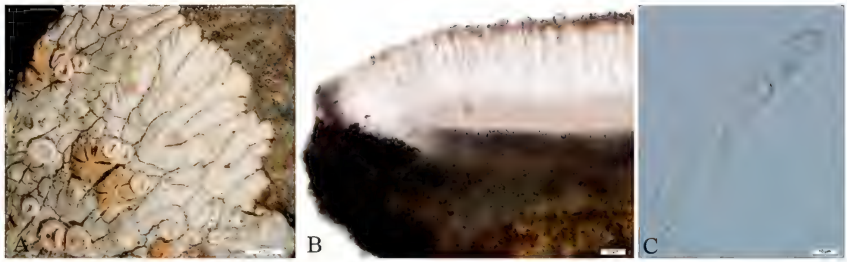

FIGURE 3. *Placopsis gelida* [Z.T. Zhao 20127934]. A. Thallus. B. Apothecial section. C. Asci and ascospores. Scale bars: A = 2 mm; B =50 μm; C = 10 μm.

hemispheres (Galloway 2007, Smith et al. 2009). It has been previously reported from Taiwan.

SPECIMEN EXAMINED —**CHINA. YUNNAN. DALI COUNTY**, Mt. Cang, alt. 3100 m, on bark, 22 Nov. 2009, Z.T. Zhao 20127934 (SDNU).

COMMENTS —*Placopsis gelida* is characterized by the matte upper surface, pale eroded soralia, deeply lobed cephalodia, and gyrophoric acid as the only major substance. The species is similar to *P. lambii*, which can be separated by its shiny surface, non-lobate cephalodia, and soralia that are usually blackish, sometimes capitate, and greenish (Galloway 2007).

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Nomenclatural notes on the lichen genera *Leucodecton* and *Myriotrema* (*Graphidaceae*) in India

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Abstract —Nine species of *Leucodecton* and nine species of *Myriotrema* are recorded for India. The recently resurrected *Leucodecton* accommodates species earlier placed in *Leptotrema*, *Myriotrema,* or *Thelotrema,* which were segregated based primarily on medullary chemistry such as stictic and norstictic acids, although *Myriotrema* retains species with a prosoplectenchymatous cortex and psoromic or protocetraric acid chemosyndromes. This contribution includes new synonyms, changes in generic placement, new species distributions, and new records. *Leucodecton compunctum* and *L. subcompunctum* are new records for India.

Key words — thelotremoid, lepadinoid, taxonomy, delimitation, tropical, Western Ghats

Introduction

The evolving taxonomic and phylogenetic studies on crustose lichens have resolved numerous worldwide nomenclature fluxes and revealed many traditionally classified families as poly- or paraphyletic. This has improved the generic delimitation of *Graphidaceae,* the largest and core tropical microlichen family (Hale 1974, 1978, 1980, 1981; Frisch 2006; Staiger 2002; Rivas Plata et al. 2012). Rivas Plata et al. (2010) revised ca. 16 genera of thelotremoid *Graphidaceae* and provided a comprehensive account of more than 260 species excluding taxa of the *Ocellularia–Myriotrema–Stegobolus* clade.

Singh & Sinha (2010) cited 130 thelotremoid lichen species from India, of which the core group includes *Chapsa*, *Chroodiscus*, *Diploschistes*, *Fibrillithecis*, *Leptotrema*, *Leucodecton*, *Melanotrema*, *Myriotrema*, *Nadvornikia*, *Ocellularia,* *Reimnitzia*, *Stegobolus,* *Thelotrema*, *Topeliopsis,* and *Wirthiotrema*. Species delimitation within the genera is based on various morpho-anatomical

differences represented by thallus and apothecial morphology, photobiont, presence or absence of lateral paraphyses/fibrils and columella, ascospore color, excipulum anatomy and carbonization, substratum, and crystal arrangement (Frisch et al. 2006; Mangold et al. 2009; Rivas Plata et al. 2010).

Based on recent collections and the available literature (Nagarkar et al. 1986, 1987, 1988; Patwardhan & Kulkarni 1977a,b; Patwardhan & Nagarkar 1980; Patwardhan & Makhija 1980; Patwardhan et al. 1985; Sethy et al. 1987; Awasthi 1991, 2000; Frisch et al. 2006; Mangold et al. 2009; Rivas Plata et al. 2010; Singh & Sinha 2010), the present study provides a synopsis of the systematics, taxonomy, and distribution pattern of the lichen genera *Leucodecton* and *Myriotrema* in India according to the current generic classification within the *Graphidaceae*.

Materials & methods

We examined and re-determined specimens from Andaman & Nicobar Islands, Andhra Pradesh, Assam, Kerala, Orissa, Tamil Nadu and West Bengal that were stored in the herbarium of the National Botanical Research Institute (LWG). We also studied recent samples collected by one author (Haridas) from different localities in Western Ghats, India. Morpho-anatomical characters were studied using a Labomed Digizoom stereomicroscope and Leica DM500 light microscope. Thin hand-cut sections of apothecia and thallus were mounted in plain water, cotton blue, 10% KOH, and iodine solutions and observed under a compound microscope. For secondary metabolites identification spot tests, calcium hypochlorite (C) and paraphenylenediamine (PD) were also applied when needed. TLC (solvent system A & C) was performed following Orange et al. (2001).

We assembled preliminary information on Indian thelotremoids from the literature (Nagarkar et al. 1986, 1987, 1988; Patwardhan & Kulkarni 1977a,b; Patwardhan & Nagarkar 1980; Patwardhan & Makhija 1980; Patwardhan et al. 1985; Sethy et al. 1987; Awasthi 1991, 2000; Singh & Sinha 2010). Earlier generic concepts and identification keys were revised according to recent world monographs based on modern concepts and classification (Frisch et al. 2006; Mangold et al. 2009; Rivas Plata et al. 2010).

Taxonomy

Leucodecton A. Massal., Atti Reale Ist. Veneto Sci. Lett. Arti, ser. 3, 5: 325, 1860.

The recently resurrected genus *Leucodecton*, originally introduced for a single species, *L. compunctum*, currently contains ca. 19 species worldwide. This includes five species each for Africa (Frisch 2006) and Australia (Mangold et al. 2009) and six from India, with one (*L. tarmuguliense*) apparently endemic for the country (Singh & Sinha 2010), although additional sampling is needed to confirm the endemism of this species. By adding three more taxa, our re-evaluation of the Indian thelotremoids brings to nine the number of *Leucodecton* species represented in the country. Taxonomic and systematic revision of the

thelotremoid *Graphidaceae* has broaden the generic delimitations so that the genus now accommodates several stictic and norstictic acid-containing species earlier placed in *Leptotrema*, *Myriotrema,* and *Thelotrema*. *Leptotrema* is separated from *Leucodecton* based on its apically thickened asci and thick-walled ascospores, while *Myriotrema* has parallel hyphae with radiating tips in the proper exciple and belongs to a separate molecular clade (Frisch et al. 2006). *Thelotrema,* on the other hand, acquires a periphysoidal excipulum. *Leucodecton* has been re-introduced to accommodate species characterized by apothecioid ascomata and a ± free proper exciple of distinctly paraplectenchymatous hyphae lacking radially oriented tips, markedly interwoven and often scarcely branched paraphyses, absence of lateral paraphyses, ± muriform brown usually I– ascospores, and presence of stictic or norstictic acids (Frisch 2006; Mangold et al. 2009; Rivas Plata et al. 2010).

Key to *Leucodecton* species recorded from India

1a. Ascospores 10–50 µm long, (2–)4–8 per ascus. 2
1b. Ascospores >50 µm long, 1–4 per ascus 6

2a. Norstictic acid present . . . . . ***L. occultum***
2b. Stictic acid present 3

3a. Apothecia often aggregated in whitish pseudostromata, thallus ecorticate . . . . . ***L. glaucescens***
3b. Apothecia solitary (sometimes numerous), thallus corticate (loose to irregular or prosoplectenchymatous) 4

4a. True cortex present, thallus hard to crystalline, ascospores 15–27(–33) µm long . . . . . ***L. compunctum***
4b. True cortex absent, thallus loose, ± dull, ascospores 20–40 µm long. 5

5a. Apothecia lepadinoid with free excipulum . . . . . ***L. subcompunctum***
5b. Apothecia myriotremoid, with ± fused excipulum . . . . . ***L. fissurinum***

6a. Ascospores remaining hyaline . . . . . ***L. anamalaiense***
6b. Ascospores brown on maturity. 7

7a. Apothecia myriotremoid to ocellularioid, thallus with irregular clusters of crystals. . . . . ***L. tarmuguliense***
7b. Apothecia porinoid to myriotremoid, thallus often with large columnar clusters of crystals. 8

8a. Apothecia porinoid (to indistinctly myriotremoid or lepadinoid with an apically free exciple), narrow pore surrounded by dark ring . . . . . ***L. compunctellum***
8b. Apothecia myriotremoid with narrow pore, thallus with small ± irregularly dispersed crystals, narrow pore surrounded by white ring. . . . . ***L. nuwarense***

Leucodecton anamalaiense (Patw. & C. R. Kulk.) Rivas Plata & Lücking, Lichenologist 42: 184, 2010.

Patwardhan & Kulkarni (1977b) first described this species from Anamalai hills in Kerala as *Thelotrema anamalaiense*. However the lepadinoid apothecia, brownish excipulum lacking lateral paraphyses, 2–4-spored asci, muriform hyaline ascospores of 50–80 × 15–20 µm, and presence of stictic acid chemosyndrome agree well with *Leucodecton,* to which it was recently transferred. The closely related *L. nuwarense* differs only in small ascospores that turn brown at late maturity (Rivas Plata et al. 2010).

Leucodecton compunctellum (Nyl.) Frisch, Biblioth. Lichenol. 92: 155, 2006.

= *Leptotrema microglaenoides* (Vain.) Zahlbr., Cat. Lich. Univers. 2: 637, 1923

= *Leptotrema elachistoteron* (Leight.) Patw. & C. R. Kulk, Norweg. J. Bot. 24: 128, 1977

= *Leptotrema oligosporum* (Müll. Arg.) Patw. & Makhija, Bryologist 83: 368, 1980

= *Myriotrema elachistoteron* (Leight.) Hale, Mycotaxon 11: 133, 1980

= *Myriotrema reclusum* (Leight.) Hale, Mycotaxon 11: 135, 1980

The species is diagnosed by pale yellowish-grey to straw colored, finely rugose to verrucose surface thallus with large columnar clusters of crystals, a fused proper exciple, porinoid apothecia with very small pores (ca. 0.05 mm), 1–4-spored asci, and brown ascospores up to 140 µm long.

Leucodecton compunctellum has previously been reported from India as *Leptotrema elachistoteron*, *L. microglaenoides*, *L. oligosporum*, *Myriotrema elachistoteron,* and *M. reclusum* (Patwardhan & Makhija 1980; Nagarkar et al. 1986; Awasthi 1991; Singh & Sinha 2010; Mangold et al. 2010). Its distribution ranges from Andaman Islands to Karnataka and Kerala.

Specimens examined—**INDIA. Andaman & Nicobar Islands:** Unknown Island, near evergreen forest compound, on bark, 23.04.1961, A. Singh 79745 (LWG); **Kerala:** Idukki district, I.C.R.I. campus, Myladumpara, alt. ca. 1200 m, on bark of tree, 01.03.1984, D.D. Awasthi & G. Awasthi 84.116 (LWG).

Leucodecton compunctum (Ach.) A. Massal., Atti Inst. Veneto Sci. lett., Arti, ser. 3, 5: 325, 1860. Plate 1a

Thallus corticolous, epiphloeodal, greenish-grey to yellowish-green or olive-green, glossy, crystalline, smooth, continuous, 114–142 µm thick. True cortex well developed, 15–25 µm thick. Prothallus indistinct, brownish. Photobiont layer inspersed with crystals largely dispersed to aggregate. Medulla indistinct to endophloeodal. Apothecia solitary, immersed, perithecioid, round, small to ca. 1.0 mm in diam. Pore minute, round, blackish, 0.2–0.3 µm in diam. Margin thick, entire, often brighter than the thallus. Disc not visible from above. Proper exciple, pale yellowish to brownish, laterally 20–25 µm reaches up to 62(–80) µm in the bottom. Epihymenium hyaline, without granules, 10–20 µm high. Hymenium clear, 150–200 µm high. Paraphyses simple, ±straight, delicate, conglutinate apically, 1–2 µm thick. Asci 8-spored, clavate, 50–70 × 15–20 µm.

Ascospores becoming brown at early maturity, muriform, 6–13 × 4–6 loculate, oblong to subglobose, 23–27(–33) × 11–14(–16) µm in size, endospore thick, I–.

Chemistry: K+ reddish, C–, PD+ orange; stictic acid chemosyndrome detected in TLC.

Ecology & distribution: Worldwide, *L. compunctum* is widely distributed in the tropics, although not recorded from Africa (Frisch et al. 2006). Newly recorded for India, it is known from tropical evergreen forests of Western Ghats above 1000 m, spreading in large thallus patches closely associated with *L. subcompunctum* and *Wirthiotrema glaucopallens* (Nyl.) Rivas Plata & Kalb.

Specimen examined— **INDIA. Kerala:** Kollam district, Rosemala, on bark, 27.06.2006, Biju Haridas 06-009582 (LWG).

Remarks: *Leucodecton compunctum* is characterized by a rather glossy smooth to crystalline thallus, immersed perithecioid apothecia with minute pores, and brown muriform ascospores. It is readily distinguished from *Leptotrema wightii* (Taylor) Müll. Arg. in lacking red anthraquinone crystals and from *Leucodecton compunctellum* and *L. subcompunctum* in having smaller ascospores. Moreover, *L. subcompunctum* has lepadinoid apothecia. *Leucodecton compunctum* may sometimes be confused with *Wirthiotrema glaucopallens* excluding pore structures; *W. glaucopallens* is further distinguished by a *Myriotrema*-type prosoplectenchymatous cortex with internal splitting (Rivas Plata et al. 2010).

Leucodecton fissurinum (Hale) Frisch, Biblioth. Lichenol. 92: 156, 2006.

A regularly fissured areolate thallus, compact surface, fused to incompletely free proper exciple bordering the rounded pore as a brownish ring, and myriotremoid apothecia are the major characters separating *L. fissurinum* from the closely related *L. subcompunctum*. Further differences are the 8-spored asci and brown muriform (3–10 × 1–3 septate) ascospores of 20–40 × 9–13 µm.

The species has been recorded at higher altitudes from Africa (Frisch 2006) and Sri Lanka (Hale 1981). In India this species was reported as *Myriotrema fissurinum* (Awasthi 1991; Singh & Sinha 2010) and is distributed mostly in the south, including Karnataka, Kerala, Tamil Nadu, and West Bengal.

Specimens examined—**INDIA. Kerala:** Idukki district, I.C.R.I. campus, Myladumpara, alt. ca. 1200 m, on bark, 01.03.1984, D.D. Awasthi & G. Awasthi 84.103 (LWG-LWU); **Tamil Nadu:** Nilgiri Hills, Avalanche, Hatchery Shola, alt. ca. 2100 m, on bark, 23.12.1971, K.P. Singh 71.564 (LWG-LWU); **West Bengal:** Eastern Himalaya, Darjeeling district, Rangit river valley, Rangit, near the bridge, alt. ca. 600 m, on bark, 08.03.1967, D.D. Awasthi & M.R. Agarwal 67.185 (LWG-LWU).

Leucodecton glaucescens (Nyl.) Frisch, Biblioth. Lichenol. 92: 164, 2006.

This taxon can easily be recognized by the chroodiscoid-lepadinoid apothecia aggregated in whitish pseudostromata, ± free proper exciple, 8-spored asci, and small brown muriform ascospores mostly under 20 × 10 µm.

Awasthi (1991) reported the species as *Myriotrema glaucescens*. The closely related *L. phaeosporum*, *L. subcompunctum,* and *L. fissurinum* have a loosely corticate thallus, mostly solitary apothecia, and comparatively larger ascospores. *L. glaucescens* occurs in tropical forests of Karnataka, Kerala, and Tamil Nadu.

Specimens examined— **INDIA. Kerala:** Palghat district, M.C.L. mines area, Walayar forest, alt. ca. 300 m, on bark, 22.03.1985, D.D. Awasthi, R. Tewari & R. Mathur 85.35, 85.3 (LWG-LWU); Idukki district, Munnar, Rajamallay area, alon border of tea plantation, alt. ca. 1500–1600 m, on bark, 24.03.1985, D.D. Awasthi, R. Tewari & R. Mathur 85.55 (LWG-LWU); on way Myladumpara to Munnar, Chinnakanal area, alt. ca. 1350 m, on bark, 02.03.1984, D.D. Awasthi & G. Awasthi 84.251 (LWG-LWU).

Leucodecton nuwarense (Hale) Frisch, Biblioth. Lichenol. 92: 155, 2006.

The species is recognized by a pale greenish-grey uneven cracked thallus containing stictic acid chemosyndrome, numerous immersed apothecia, free proper exciple, 2-spored asci, and brown muriform ascospores of 42–55 × 10–15 µm.

Originally included in Indian thelotremoid group as *Leptotrema nuwarense* and *Myriotrema nuwarense* from South Andaman (Nagarkar et al. 1986; Awasthi 1991), *L. nuwarense* is close to *L. anamalaiense,* which is distinguished by consistently hyaline and somewhat larger ascospores.

Leucodecton occultum (Eschw.) Frisch, Biblioth. Lichenol. 92: 157, 2006.

= *Leptotrema compunctum* (Ach.) Nyl., Flora 71: 527, 1888

= *Leptotrema norstictideum* Patw. & Nagarkar, Biovigyanam 6(1): 5, 1980

This species is described for an ecorticate fissured thallus in different shades of pale and yellow (greenish or grey), immersed to slightly emergent ascomata with a free proper exciple, small brown submuriform or muriform (5–9 × 1–5 septate), thick-walled ascospores (20–40 × 10–17 µm), and presence of norstictic acid chemosyndrome.

Awasthi & Singh (1975) and Patwardhan & Kulkarni (1977a) cited *L. occultum* as *Leptotrema compunctum*, and Patwardhan & Nagarkar (1980) as *Leptotrema norstictideum*. In India the species is found in the Andaman and Nicobar Islands, Andhra Pradesh, Assam, Kerala, Madhya Pradesh, Meghalaya, Orissa, Tamil Nadu, and West Bengal. It is the only norstictic acid (major) containing species of *Leucodecton*.

Specimens examined—**INDIA. Andhra Pradesh:** Vishakhapatnam, Simhachadam area in cashew plantation, on bark of Mango tree, 06.03.1986, D.D. Awasthi, G. Awasthi, R. Mathur & P. Srivastava 86.240, 86.248 (LWG-LWU); **Kerala:** Trivandrum Botanical and Zoological Garden, on bark, 08.05.1979, D.D. Awasthi, D.K. Upreti & U. Mishra 79.898 (LWG-LWU); **Orissa:** Ganjam district, on way to Seranga, on bark of *Pongamia* tree, 03.03.1986, D.D. Awasthi, G. Awasthi, R. Mathur & P. Srivastava 86.128 (LWG-LWU); **West Bengal:** 24 Parganas district, Sunderban, Sajanakholi forest office, on bark, May 1975, K. N. Roychowdhury 3871, 3862, 3875, 3879 (LWG-CAL).

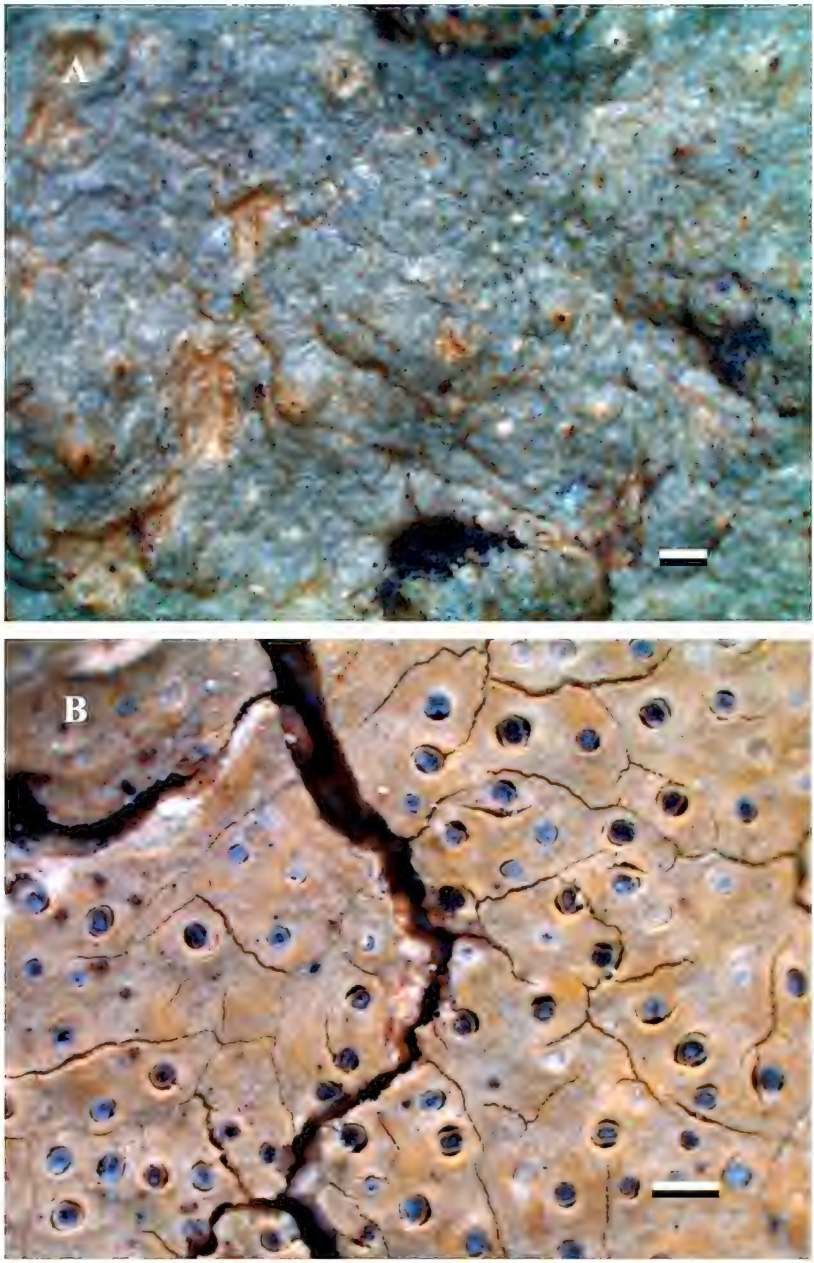

Plate 1. New *Leucodecton* records from India.
A. *Leucodecton compunctum*. B. *Leucodecton subcompunctum*.
Scale bars: A = 1 mm; B = 2 mm.

Leucodecton subcompunctum (Nyl.) Frisch, Biblioth. Lichenol. 92: 162, 2006.

PLATE 1B

Thallus corticolous, epiphloeodal, olive-grey to olive-brown, fissured to areolate, ± glossy, loose, irregularly corticate, crystalline surface. Prothallus brownish. Photobiont layer well developed, with inclusions of calcium oxalate crystals, up to 50 µm thick. Medulla largely endophloeodal. Apothecia dispersed, immersed, rounded to angular, up to 1.0 mm wide pore. Disc blackish, usually covered with white pruina. Proper exciple free to partly fused, pale fawn to sometimes hyaline, 15–25 µm thick. Epihymenium unpigmented, up to 10 µm high. Hymenium clear, 100–120 µm high. Subhymenium 10–20 µm high. Paraphyses simple, straight, 1.5–2.0 µm thick, tips slightly thickened, adspersed with grayish granules. Asci 8-spored, narrowly clavate, 80–100 × 10–20 µm in size. Ascospores brown, muriform, 3–8 ×1–3 septate, 15–35 × 10–20 µm in size, I–.

CHEMISTRY: K+ reddish, PD+ orange, C–. Stictic acid chemosyndrome detected in TLC.

ECOLOGY & DISTRIBUTION: Previously known from tropical, sub-tropical to temperate regions in Africa, Asia, and Australia (Frisch 2006), *L. subcompunctum* is reported for the first time from India from the tropical evergreen forests of Western Ghats (Kerala and Tamil Nadu). It can grow in shade but is found mostly in open sub-temperate forests and avoids humid environment.

SPECIMENS EXAMINED— **INDIA. KERALA:** Idukki district, Kallar Munnar Hills, alt. 1140 m, on bark, 14.02.1975, A. Singh & M. Ranjan 103044 (LWG); Trivendrum, ABP, way to Pongalappara, alt. 1150 m, on bark, 26.04.2006, Biju Haridas 06–009580 (LWG); **TAMIL NADU:** Nilgiri Hills, Kodanad, tea estate area, alt. ca. 2019 m, on bark, 31.12.1970, D.D. Awasthi & K.P. Singh 70.1447, 70.1500 (LWG-LWU); In the shola near tea estate, alt. ca. 2010 m, on bark, 31.12.1970, D.D. Awasthi & K.P. Singh 70.1499 (LWG-LWU); Salem district, Yercaud, Shevaroy Hills, near Shevaroy temple, alt. 1600 m, on bark, 29.12.1990, D.K. Upreti & Hariharan 202308 (LWG); Palni Hills, Kodaikanal, near Shola, alt. ca. 1800 m, on bark, 13.12.1970, K.P. Singh, 70.849 (LWG-LWU).

REMARKS: Frisch (2006) considered *L. subcompunctum* a lowland species and separated it from the customarily high elevation *L. fissurinum* based on altitudinal range and certain minor diagnostic differences. But the recent field survey together with the re-examination of preserved material indicates that in India *L. subcompunctum* occurs at significantly higher altitudes in montane forests. However, the compact crystalline irregularly fissured to areolate thallus and lepadinoid apothecia with free excipula separate *L. subcompunctum* from *L. fissurinum*. *Leucodecton phaeosporum* (Nyl.) Rivas Plata & Lücking, which resembles *L. subcompunctum* in having a loose and irregular cortex, differs in columnar crystal arrangement in the thallus and comparatively small ascospores of 15–25 µm.

Leucodecton tarmuguliense (Sethy, Nagarkar & Patw.) Frisch, Biblioth. Lichenol. 92: 155, 2006.

The species is characterized by a pale yellowish-grey to olivaceous thallus containing stictic acid chemosyndrome, prominent apothecia, a fused proper exciple, 2-spored asci and brown muriform ascospores of 70–93 × 15–23 µm. Sethy et al. (1987) described this species as *Leptotrema tarmuguliense* from south Andaman, which was accommodated in *Myriotrema* (Awasthi 1991) until the recent thelotremoid taxonomic reclassification. The species is well separated from the closely related *L. compunctellum* by its prominent (emergent) apothecia and irregular clusters of crystals. An Indian endemic, *L. tarmuguliense* is reported thus far only from Andaman and Nicobar Islands.

Myriotrema Fée, Essai Crypt. Écorc. 1: xlix, 103, 1825.

The genus, recognized as a section in *Thelotrema* (Salisbury 1978), was reestablished by Hale (1980, 1981) to accommodate species that lack lateral paraphyses and have non-carbonized proper exciples. Frisch et al. (2006) later revised *Myriotrema* for six species in Africa and Mangold et al. (2009) for 17 species in Australia. Worldwide ca. 25 *Myriotrema* species were recognized prior to emendation by Rivas Plata et al. (2010), who circumscribed the genus to emphasize the internally splitting prosoplectenchymatous cortex and production of psoromic and protocetraric chemosyndromes. In India the genus was represented by 24 species (Awasthi 1991, 2000), subsequently reduced to 15, including three endemics (Singh & Sinha 2010). Due to the most recent generic classification, most *Myriotrema* taxa have been transferred to *Chapsa, Leucodecton*, *Ocellularia, Thelotrema,* and *Wirthiotrema,* with some reduced to synonymy. Currently *Myriotrema* is represented in India by nine species (including one endemic) featuring psoromic acid as a major chemical constituent with some species containing protocetraric acids and unknown compounds. Other diagnostic characters include an internally splitting prosoplectenchymatous cortex, small myriotremoid apothecia, prosoplectenchymatous proper exciples with radiating tips, and an absence of lateral paraphyses.

Key to *Myriotrema* species recorded from India

1a. Ascospores muriform to submuriform 2
1b. Ascospores transversely septate (sometimes with 1 vertical septum) 5
2a. Ascospores brown ..... ***M. desquamans***
2b. Ascospores hyaline 3
3a. Lichen substances absent ..... ***M. subconforme***
3b. Lichen substances present (psoromic acid) 4

4a. Asci 4–8-spored, ascospores small, 15–25 × 7–10 µm ..... ***M. rugiferum***
4b. Asci 1-spored, ascospores large, 140–240 × 24–50 µm ..... ***M. masonhalei***
5a. Thallus containing protocetraric acid present ..... ***M. pertusarioides***
5b. Thallus lacking protocetraric acid 6
6a. Apothecia usually emergent, psoromic acid chemosyndrome ..... ***M. glaucophaenum***
6b. Apothecia immersed to raised, chemistry variable 7
7a. Thallus containing olivaceum unknown ..... ***M. olivaceum***
7b. Thallus containing psoromic acid chemosyndrome 8
8a. Thallus glossy, ± free proper exciple, ascospores usually with 1 vertical septum..... ***M. clandestinum***
8b. Thallus fissured, areolate, ± fused proper exciple, ascospores consistently transversely septate ..... ***M. microporum***

Myriotrema clandestinum (Fée) Hale, Mycotaxon 11: 133, 1980.
= *Ocellularia terebratula* (Nyl.) Müll. Arg., Mém. Soc. Phys. Genève 29(8): 12, 1887

The species is characterized by pale greenish to olivaceous corticate continuous glossy thallus, immersed apothecia, ± free proper exciple, small hyaline transversely 5–6-septate (with one vertical septum) ascospores of 10–27 × 6–8 µm, and the presence of psoromic acid.

Myriotrema clandestinum, reported from India by Nagarkar et al. (1988) as *Ocellularia terebratula*, has been included in the synonymy of *Myriotrema clandestinum* by Mangold et al. (2009). It has a wide distribution in India and collected from Andaman & Nicobar Islands, Arunachal Pradesh, Karnataka, Kerala, Maharashtra, and Meghalaya.

Specimens examined—**INDIA. Kerala:** Mallapuram district, Wynad area, Thakarpadi, A. Singh & M. Ranjan, alt. 450 m, on bark 102217 (LWG); Trivendrum Peppara Wildlife Sanctuary, on bark, 19.10.2006, Biju Haridas, 06–009586 (LWG); Kollam, Rosemala, 27.6.2006, on bark, B. Hardas, 06–009585 (LWG); **Tamil Nadu:** Palni Hills, Perumal to Palni road side, via short-cut road, alt. 1350–1500 m., on bark, 15.12.1970, K.P. Singh 70.984 (LWG).

Myriotrema desquamans (Müll. Arg.) Hale, Mycotaxon 11: 133, 1980.
= *Leptotrema irosinum* (Vain.) Zahlbr., Cat. Lich. Univ. 2: 635, 1923

The taxon is characterized by a glossy corticate thallus, small-pored immersed perithecioid ascomata, a fused proper exciple, and brown muriform (5–11 × 1–6 septate) ascospores of 20–35 × 8–18 µm.

In India the species was reported as *Leptotrema desquamans* by Patwardhan & Makhija (1980) and *L. irosinum* by Patwardhan & Kulkarni (1977a). The species shows close affinity with *Wirthiotrema* and forms a non-inspersed counterpart of *W. trypaneoides* (Nyl.) Rivas Plata & Lücking (Rivas Plata et al.

2010). In India the species is known from Andaman & Nicobar Islands and Kerala.

Myriotrema glaucophaenum (Kremp.) Hale, Mycotaxon 11: 133, 1980.

The greenish to yellowish-grey thallus, ± free proper exciple, emergent apothecia, 8-spored asci, transversely 1–7 septate (with single vertical septum) hyaline ascospores of 10–20 × 5–8 µm, and presence of psoromic acid are the characteristic features of the species. The closely related *M. microporum* and *M. clandestinum* with psoromic acid differs in having a fissured thallus and a fused proper exciple, respectively. *Myriotrema glaucophaenum* is unusual in its *Thelotrema*-like ascomata that lack lateral paraphyses (Mangold et al. 2009). Patwardhan & Kulkarni (1977a) reported the species from Kerala as *Ocellularia glaucophaena.*

Specimen examined—**INDIA. Kerala:** Trivendrum, ABP, Athirumala Pathalamathy, alt. 1200 m, on bark, 25.04.2006, Biju Haridas 06–009587 (LWG).

Myriotrema masonhalei (Patw. & C. R. Kulk.) Hale, Mycotaxon 11: 134, 1980.

The species is diagnosed by the dark greenish glaucous, wrinkled to warty thallus, immersed apothecia, a pale-brown fused proper exciple carbonized at apices, 1-spored asci, and oblong-ellipsoid, large (140–240 × 24–50 µm) ascospores.

This psoromic acid containing species is described from Maharashtra by Patwardhan & Kulkarni (1977) as *Thelotrema masonhalei* and has also been reported from Kerala and Karnataka in Western Ghats in India (Singh & Sinha 2010).

Myriotrema microporum (Mont.) Hale, Mycotaxon 11: 134, 1980.

The taxon is readily recognized by a thick areolate fissured dark dull-grey thallus, numerous small apothecia, a ± fused proper exciple, 8-spored asci, transversely 2–4-septate hyaline ascospores of 10–18 × 5–8 µm, and the presence of psoromic acid chemosyndrome.

Myriotrema microporum shares the same chemical constituents with *M. clandestinum* and *M. glaucophaenum* but differs in the distinctly fissured, areolate thallus and slightly small transversely septate ascospores. Earlier it was reported as *Ocellularia micropora* from Assam, Karnataka, Kerala, Orissa, Tamil Nadu, and West Bengal (Nagarkar et al. 1988, Singh & Sinha 2010). The species is found in Western Ghats, eastern Himalayas, and Eastern Ghats in India.

Specimens examined—**INDIA. Assam:** North Cachar Hills district, Haflong, on bark, D.K. Upreti & Jayshree Rout 05–002992 (LWG); **Kerala:** Idukki district, I.C.R.I. campus, Myladumpara, alt. ca. 1200 m, on bark, 01.03.1984, D.D. Awasthi & G. Awasthi 84.118, 84.148 (LWG-LWU); on bark of *Vateria indica*, 01.03.1984, D.D. Awasthi & G. Awasthi 84.128 (LWG-LWU); Myladumpara to Munnar, Chinnakanal area, alt. ca.

1350 m, on bark, 02.03.1984, D.D. Awasthi & G. Awasthi 84.228, 84.248 (LWG-LWU); Santhampara area, alt. ca. 1200 m, on bark, 02.03.1984, D.D. Awasthi & G. Awasthi 84.177 (LWG-LWU); Kallar Munnar Hills, alt. 1140 m, on bark, 14.02.1975, A. Singh & M. Ranjan 103065 (LWG); Travancore, Quilon, on bark, 10.08.1953, O.A. Höeg 2580 (LWG-AWAS); **Orissa:** Ganjam district, on way to Seranga, on bark of *Pongamia* tree, 03.03.1986, D.D. Awasthi, G. Awasthi, R. Mathur & P. Srivastava 86.127 (LWG-LWU); **Tamil Nadu:** Palni Hills, Perumal to Palni road side, via short cut road, alt. 1350–1500 m, on bark, 15.12.1970, K.P. Singh 70.981 (LWG-LWU); Tiger Shola area, along the road, alt. ca. 1650 m, on bark, 15.12.1970, K.P. Singh 70.1028 (LWG); Shembaganur to Periakulum, via short cut road, alt. 1650–1800 m, on bark, 14.12.1970, K.P. Singh 70.935 (LWG-LWU).

Myriotrema olivaceum Fée, Essai Crypt. Écorc. 1: 103, 1825.

The taxon is distinguished by the thallus compound "olivacea unknown," a light greenish-grey to ashy grey smooth fissured areolate thallus, free proper exciple, 8-spored asci, and small (10–15 × 5–7 µm) transversely 2–4-septate ascospores.

The unusual chemical compounds separate the species from the closely related *M. microporum*, which has similar apothecial morpho-anatomical characters. Nagarkar et al. (1986) reported *M. olivaceum* (as *Ocellularia olivacea*) from Andaman and Nicobar Islands, the only sites reported for India.

Myriotrema pertusarioides (Nagarkar, Sethy & Patw.) D.D. Awasthi, Biblioth. Lichenol. 40: 3, 1991.

The species is characterized by a pale to yellowish-grey warty thallus, numerous emergent basally constricted apothecia, a fused reddish-yellow proper exciple, 8-spored asci, and 15–18 × 10–12 µm ascospores with single transverse and vertical septa.

Nagarkar et al. (1986) described this protocetraric acid-containing species (as *Leptotrema pertusarioides*) from the Andaman and Nicobar Islands, where it appears endemic. However, further taxonomic study is needed, as its apothecial morphology is unusual for the genus, and it resembles *Ocellularia bahiana* (Ach.) Frisch in having protocetraric acid.

Myriotrema rugiferum (Harm.) Hale, Mycotaxon 11: 135, 1980.

This morphologically variable taxon is characterized by a pale-olive to greyish and greenish-white thick corticate thallus, small immersed apothecia, a free proper exciple, 4–8-spored asci, and small (15–25 × 7–10 µm) hyaline submuriform (3–6 × 0–3 septate) ascospores. Similar to *M. masonhalei*, *M. rugiferum* contains psoromic acid but differs in having 1-spored asci and large ascospores. *Myriotrema rugiferum* has been collected only from tropical forests of Kerala in India.

Specimens examined—**INDIA. Kerala:** Idukki district, I.C.R.I. campus, Myladumpara, alt. ca. 1200 m, on bark, 01.03.1984, D.D. Awasthi & G. Awasthi 84.136, 84.73, 84.83 (LWG-LWU).

Myriotrema subconforme (Nyl.) Hale, Mycotaxon 11: 135, 1980.

Distinguishing features include a pale-olive to greyish-green or greenish-grey verruculose rather fragile corticate thallus with numerous crystals inclusions, a free proper exciple, 8-spored asci, and small (10–20 × 6–9 µm) submuriform (3–5 × 1–3 septate) ascospores.

Myriotrema subconforme appears most closely related to *M. album* Fée, *M. clandestinum*, *M. myrioporum* (Tuck.) Hale, and *M. endoflavescens* Hale ex Lücking in lacking thallus compounds. However, all three species differ from *M. subconforme* in their mostly transversely septate ascospores, and *M. endoflavescens* has a yellow-pigmented medulla (Lumbsch et al. 2011). The submuriform species, *M. rugiferum*, contains psoromic acid and has slightly larger (ca. 0.4 mm) apothecia (Mangold et al. 2009).

Nagarkar et al. (1987) reported *M. subconforme* (as *Thelotrema subconforme*) from Andaman and Nicobar Islands. Reexamination of certain unidentified preserved specimens from Kerala has extended the range of this species to Western Ghats.

Specimens examined—**INDIA. Kerala:** Palghat district, M.C. mines area, Walayer forest, alt. ca. 300 m, on bark, 22.03.1985, D.D. Awasthi, R. Tewari & R. Mathur 85.8 (LWG-LWU); Kollam district, rosemala, on bark, 28.06.2006, Biju Haridas 06–009588 (LWG); Trivendrum, ABP, way to Pongalappara, alt. 1145 m, on bark, 26.04.2006, Biju Haridas 06-009625 (LWG); **Tamil Nadu:** Nilgiri Hills, Awalanche, in Shola near forest rest house, alt. ca. 2100 m, on bark, 04.01.1971, D.D. Awasthi & K.P. Singh 71.247 (LWG-LWU).

Discussion

The *Graphidaceae* flourish well in India and offer a remarkable diversity in Andaman & Nicobar Islands, eastern Himalayas, and Western Ghats. Of the 18 taxa studied here, 14 species (seven each of *Leucodecton* and *Myriotrema*) occur in Western Ghats, followed by nine (four *Leucodecton* and five *Myriotrema*) reported from Andaman & Nicobar Islands and four species (two each of *Leucodecton* and *Myriotrema*) from eastern Himalayas. Eastern Ghats harbours *Leucodecton occultum* and *Myriotrema microporum* whereas central India is represented by *Leucodecton occultum*. Despite the variations in macro-habitat, tree species, trunk girth, bark texture, and other microclimate variables, it is noteworthy that thelotremoid lichens exhibit a uniform pattern of association with other lichens on different trees of tropical rain forests in India. Although placed in significantly different clades by molecular analysis, *Leucodecton* and *Myriotrema* especially show morphological similarities and share habitats

over a broad (300–2100 m) altitudinal range. *Leucodecton* mostly inhabits the wide tree trunks and associates with other porinoid or lepadinoid species (e.g., *Myriotrema*, *Ocellularia, Thelotrema* and *Wirthiotrema*) in comparatively dry but shaded undisturbed to semi-disturbed mature secondary lowland forests. Ecorticate taxa retain their rough texture that easily distinguishes them from neighbouring corticate taxa. However, the corticate shiny hard texture of certain *Leucodecton* taxa is so close to some *Myriotrema* and *Ocellularia* species that field identification is difficult. Superficially, the thallus and apothecial structures in some *Leucodecton* species (e.g., *L. compunctellum*) are enough similar to those in certain *Myriotrema* species that chemical tests are needed for correct determination.

Thelotremoid lichens in India are in great need of systematic revision due to remaining taxonomic and nomenclatural problems. In certain cases the taxonomically functional characters for species differentiation are not very sharp (Staiger et al. 2006, Rivas Plata & Lumbsch 2011). In particular, delimiting species based on ascospore color changing progressively from hyaline to brown during development is overemphasized, as for *Leucodecton anamalaiense*, *L. compunctellum,* and *L. nuwarense*. Differentiation between *L. compunctellum* and *L. nuwarense* is based broadly on crystal arrangement and color of the pore margins, with separation of the two dependent on rather similar characteristics of apothecia, pore structures, size, and thallus texture (Rivas Plata et al. 2010). Further, *L. fissurinum* is treated as a separate species based on such minor taxonomic features as a regularly fissured thallus and myriotremoid apothecia with ± fused exciple. The closely related *L. subcompunctum,* which also exhibits a fissured thallus, does have a diagnostic lepadinoid apothecial morphology. Likewise, *Myriotrema clandestinum* differs from *M. microporum* in its somewhat smooth thallus, ± fused proper exciple, and ascospores with a single vertical septum. The closely related *M. microporum* has a fissured thallus, ± free proper exciple, and transversely septate ascospores within the range of *M. clandestinum* (Mangold et al. 2009). Species delimitation based on vertical septum as suggested by Salisbury (1978) has little taxonomic significance. Moreover, characters like a fissured thallus, a more or less free to fused excipulum, the arrangement or abundance of crystals, or the involvement of a periderm layer in the apothecial margins (Frisch 2006; Mangold et al. 2009; Rivas Plata et al. 2010) seem to have little taxonomic importance and should be considered ecologically influenced variations. Nonetheless, it might be beneficial to know whether such morphological variations result from genetic differentiation or phenotypic plasticity (Rivas Plata & Lumbsch 2011, Rivas Plata et al 2011).

The unrevised taxa, *M. masonhalei* and *M. pertusarioides,* both with 1-spored asci, are currently placed in *Myriotrema*. Occurrence of a single spore is rare in *Myriotrema*. Furthermore, the ascospore size in *M. masonhalei* is unusually

large for *Myriotrema*, while the basally constricted emergent apothecia in *M. pertusarioides* seems more characteristic of *Ocellularia,* indicating that both species need further analysis for proper placement. Several thelotremoid taxa have uncertain positions in either molecular or traditional taxonomic classification, suggesting that they need to be split into several different taxa or delimited more broadly to resolve ambiguities at the generic or species levels.

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Three non-hairy species of *Leptogium* from China

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Abstract — *Leptogium javanicum* is reported as new to China, and *L. austroamericanum* and *L. sessile* are reported as new to mainland China. Descriptions and comments for the three species and a key to the non-hairy species known from mainland China are presented.

Key words — taxonomy, *Ascomycota*, *Peltigerales*, *Collemataceae*, lichenized fungi

Introduction

The lichen genus *Leptogium* (Ach.) Gray comprises about 189 species worldwide (Kirk et al. 2008). Of the 34 species reported for China, 22 have been recorded from the mainland (Wei 1991, Wang et al. 2010, Liu & Guan 2012). Eight out of 17 non-hairy species have been recognized in mainland China: *Leptogium azureum* (Sw. ex Ach.) Mont., *L. cochleatum* (Dicks.) P.M. Jørg. & P. James, *L. cyanescens* (Pers.) Körb., *L. denticulatum* Malme, *L. lichenoides* (L.) Zahlbr., *L. moluccanum* (Pers.) Vain., *L. plicatile* (Ach.) Leight., and *L. subazureum* A. Dube & Makhija (Aptroot et al. 2002, Wei 1991).

In this paper, three additional non-hairy species belonging to the genus are reported. *Leptogium javanicum* is new to China, and *L. austroamericanum* and *L. sessile* are new to mainland China.

Material & methods

The specimens studied are housed in the Herbarium of Nanjing Normal University (NNU), the Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica (KUN-L), and Lichen section of Herbarium of Mycology, Academia Sinica (HMAS-L). A dissecting microscope (Motic SMZ-140) and a light microscope (Motic B2) were used for the morphological and anatomical studies. A microtome was routinely used for frozen sections. Photographs were taken with BX51 fluorescence microscope.

New records

Leptogium austroamericanum (Malme) C.W. Dodge, Ann. Mo. Bot. Gdn 20: 419 (1933) FIG. 1A–B

THALLUS foliose, 3–8 cm in diam; UPPER SURFACE grey-blue when dry, with distinct wrinkles, somewhat shiny; LOBES orbicular, spreading to erect, 4–10 mm broad, the margin entire to irregularly dentate to isidiate; ISIDIA terete, simple, laminal to marginal, dense, crowded; LOWER SURFACE similar to upper surface.

THALLUS 100–150 µm thick when moist, with single-layered cortex on both sides; UPPER CORTEX cells 3–5 µm in diam.; LOWER CORTEX cells 4–7 µm in diam.; PHOTOBIONT *Nostoc* in chains, spherical, 5–6 µm in diam.; hyphae irregularly interwoven.

APOTHECIA not seen.

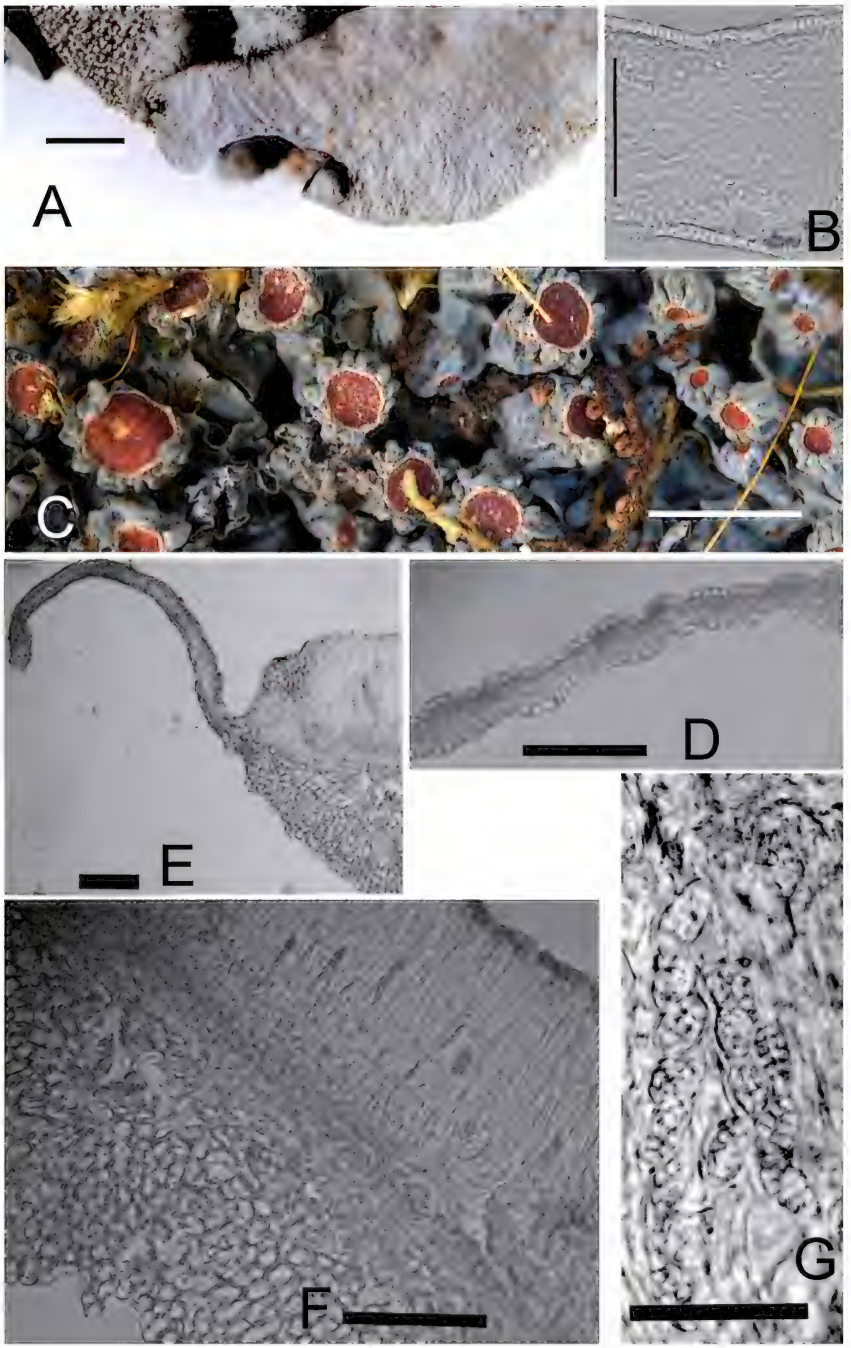
Corticolous or muscicolous.

SPECIMENS EXAMINED: CHINA. **ZHEJIANG PROVINCE**, Mt. Jiulongshan, alt. 570 m, on moss, 28 Apr. 1987, S.F. Chen Z0193 (NNU); **GUANGXI PROVINCE**, Longsheng County, on bark, 21 Aug. 1964, M. Zang 776 (NNU); **GUANGDONG PROVINCE**, Nanxiong City, alt. 700 m, on moss, 12 May 1978, A.T. Liu 780087 (NNU); **YUNNAN PROVINCE**, Yingjiang County, alt. 1200 m, on moss, 20 Jun. 1981, X.Y. Wang, X. Xiao & J.J. Su 3121 (HMAS-L: 031791).

REMARKS — *Leptogium austroamericanum* is muscicolous or corticolous in montane forests from Southeast and Southwest China at elevations of 570–1200 m. It has been reported from North America, Australia, New Zealand, India, and Taiwan (Sierk 1964, Verdon 1990, Galloway 1999, Awasthi & Akhtar 1979, Aptroot et al. 2002). It is new to mainland China.

Our materials, while similar to those reported in North America in morphology and anatomy, have thinner lobes (100–250(–300) µm in North America). They are also similar to those reported in Australia, but the latter often have squamuliform isidia that were not found in our materials. The materials in New Zealand have isidia granular at first, then become terete and simple, and are coralloid-branched when mature, while in our materials the isidia are always terete and simple. Our materials also differ from those found in India in having larger thalli (1.5–3 cm in India), thicker lobes (60–150 µm in India), and different isidia (terete and simple to branched and squamuliform in India).

FIG. 1. ***Leptogium austroamericanum*** (M. Zang 776, NNU). A. Thallus with isidia. B. Thallus cross-section, showing corticated surfaces. ***Leptogium javanicum*** (L.S. Wang 05-24176, KUN-L). C. Thallus with apothecia. D. Thallus cross-section, showing corticated surfaces. E. Apothecial cross-section. F. Vertical section of the central apothecium. G. Ascus with 8 ascospores. Scale bars: A, C = 1 cm; B, D–F = 100 µm; G = 50 µm.


A
B
C
D
E
F
G

The species is similar to *L. cyanescens* in the colour of the thallus, numerous simple isidia, and rare apothecia. However, in *L. cyanescens* the thallus is smooth, not wrinkled. Another closely related species, *L. propaguliferum*, produces flattened phyllidia instead of terete isidia.

Leptogium javanicum Mont., Syll. Gen. Sp. Crypt.: 379 (1856) FIG. 1C–G

THALLUS 4–7 cm in diam., foliose, outline irregular, crisp; UPPER SURFACE grey-blue when dry, glabrous, smooth, slightly roughened to striate, without wrinkles, dull; LOBES 3–5 mm broad, often suberect, the margin wavy, entire to irregularly lacerate, upturned or downturned; ISIDIA and LOBULES absent; LOWER SURFACE similar to the upper surface, but with sparse, brown to blackish, 1.5–3.0 mm long rhizines. APOTHECIA abundant, dense, laminal to marginal, 1–3 mm in diam., distinctly stalked, at the apex of dome-shaped, vertically furrowed (when dry) lobes; DISC plane to concave, red-brown; THALLINE EXCIPLE glabrous, concolorous with the thallus.

THALLUS 20–50 µm thick; CORTEX cells on both sides consisting of a single layer of globular, isodiametrical cells, 5–8 µm in diam.; PHOTOBIONT *Nostoc* in chains, spherical, 4–6 µm in diam.; HYPHAE irregularly interwoven.

APOTHECIA 300–350 µm thick; THALLINE EXCIPLE euparaplectenchymatous, 50–100 µm at margin, consisting of 4–6 layers of subglobose to ellipsoid cells; LOWER CORTEX below the apothecium 100–150 µm thick, euparaplectenchymatous, consisting of 7–8 layers of subglobose to ellipsoid, thin-walled cells; PROPER EXCIPLE not seen; HYMENIUM cir. 130 µm high; SUBHYMENIUM 20–30 µm high; ASCUS clavate, 100–120 × 15–25 µm, with 8 ascospores; ASCOSPORES submuriform, ellipsoid, 10–20 × 8–12 µm, 3–5 septate transversely, 1 septate longitudinally, obtuse to slightly acute at both ends.

Corticolous.

SPECIMENS EXAMINED: **CHINA. YUNNAN PROVINCE**, Pu'er City, alt. 1420 m, 18 Jan. 2005, L.S. Wang 05-24176 (KUN-L), 05-24195 (KUN-L); Menghai County, 11 Oct. 1974, X.J. Li 487 (KUN-L 3558).

REMARKS — *Leptogium javanicum* is corticolous in montane forests in Yunnan, Southwest China, at an elevation of about 1400 m. It has been reported in India (Awasthi & Akhtar 1979), Nepal, the Galapagos Islands, (Bungartz 2008), and Australia (Verdon 1992). It is new to China.

The Chinese materials are similar to those reported in the Galapagos, but with smaller ascospores (22–30 × 10–15 µm in the Galapagos), less wrinkled upper surface and furrowed apothecial stalks. It is also similar to those reported in Australia (Verdon 1992) in having furrowed, hollow stalks that attach to the upper apothecial margins, but our materials have smaller ascospores (25–35

× 13–15 μm in Australia) and less-wrinkled and thinner thalli (60–120 μm in Australia).

Leptogium javanicum can be readily recognized by its apothecia at the apex of the dome-shaped lobes and its suberect lobes. It also somewhat resembles *L. verrucosum* with respect to the thallus colour and poorly developed proper exciple. However, *L. verrucosum* has larger ascospores (15–32 × 6–12 μm) and a warty thallus owing to the presence of numerous pycnidia.

Leptogium sessile Vain., Ann. Acad. Sci. Fenn., Ser. A 6(7): 108 (1915) FIG. 2

THALLUS 2–6 cm in diam., foliose; UPPER SURFACE grey-blue when dry, with distinct wrinkles, shiny; LOBES orbicular, 5–10 mm broad, the margin entire and downturned; ISIDIA absent; LOWER SURFACE similar to upper surface. APOTHECIA common, ± dense, laminal to submarginal, sessile, 1–2 mm in diam.; DISC plane to concave, red-brown; THALLINE EXCIPLE wrinkled, concolorous with the thallus.

THALLUS 170–450 μm thick; CORTEX cells on both sides consisting of a single layer of globular, isodiametrical cells, 4–5 μm in diam.; PHOTOBIONT *Nostoc* in chains, spherical, 4–6 μm in diam.; HYPHAE irregularly interwoven.

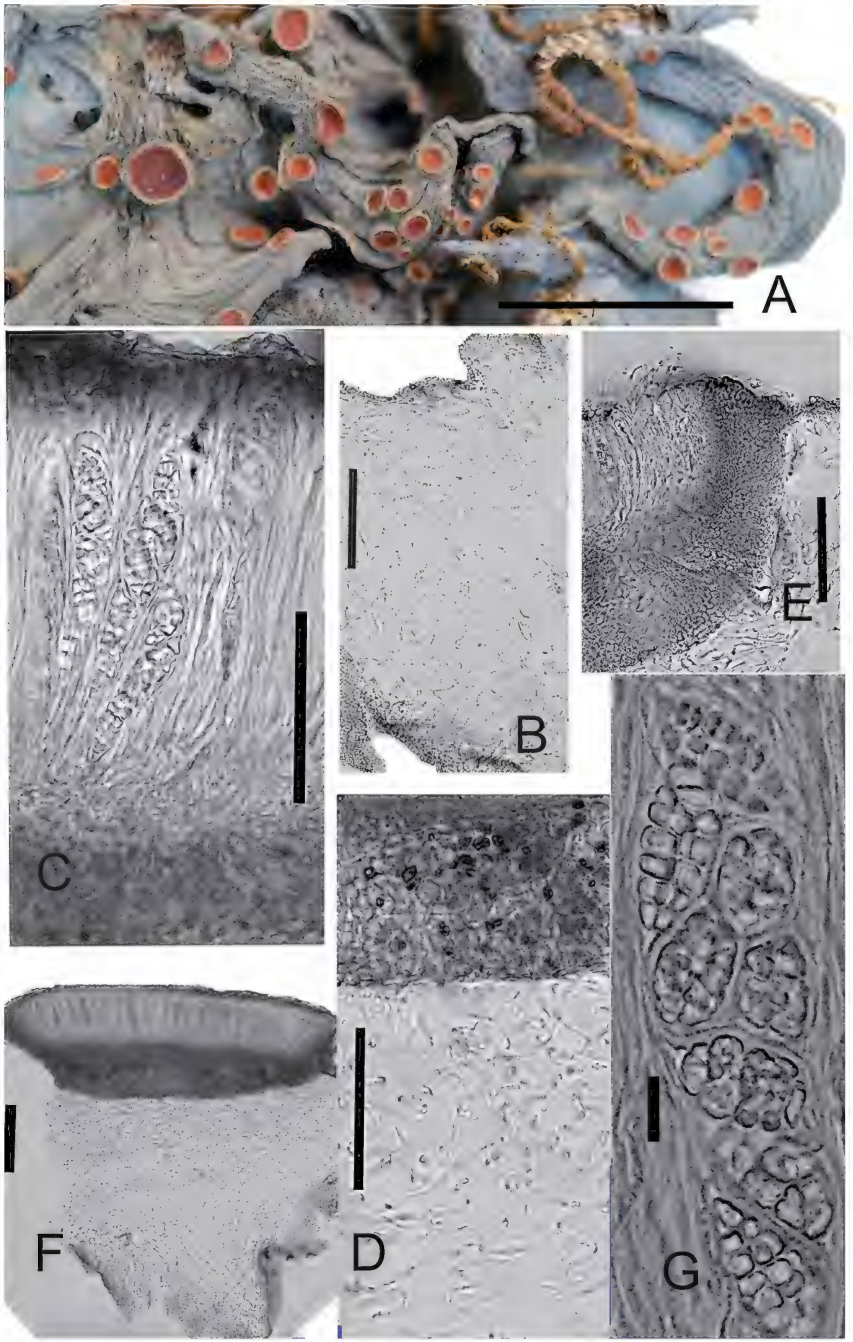
APOTHECIA 550–700 μm thick; THALLINE EXCIPLE 4–5 μm thick at margin, consisting of a single-layered, thin-walled, subglobose cells, 4–5 μm in diam., and an algal layer with loosely to somewhat densely interwoven hyphae; PROPER EXCIPLE euparaplectenchymatous, 70–120 μm thick; HYMENIUM 175–225 μm high; SUBHYMENIUM 50–70 μm high; ASCUS clavate, 100–125 × 10–15 μm; ASCOSPORES 8 per ascus, submuriform to muriform, ellipsoidal, 25–35 × 8–13 μm, 3–7 septate transversely, 1–2 septate longitudinally.

Corticolous.

SPECIMENS EXAMINED: **CHINA**. **ANHUI PROVINCE**, Mt. Huangshan, on bark, 7 Sept. 1974, J.N. Wu & T. Xiang, 740078 (NNU); **YUNNAN PROVINCE**, Lvchun County, on moss, 15 May. 1975, X.J. Li, 74-1724 (KUN-L); Ruili City, on bark, 30 Nov. 1980, Y.M. Jiang, 587-2 (HMAS-L 031787); Weixi County, alt. 2100 m, on bark, 13 July. 1981, X.Y. Wang, X. Xiao & J.J. Su, 3699 (HMAS-L 031771).

REMARKS — *Leptogium sessile* is mainly corticolous in montane forests of Southwest and Southeast China. This species has been reported from North America and Taiwan (Sierk 1964, Aptroot et al. 2002). It is new to mainland China.

Our materials are similar to those reported in North America, but with somewhat thinner lobes (300–500 μm thick in North America), and narrower ascospores (23–35 × 12–16 μm in North America). Although the North American materials have ascospores with 0–2 longitudinal septa (Sierk 1964), no such septa were found in our materials from China.


A
B
C
D
E
F
G

Leptogium sessile resembles *L. corticola* in having a distinctly wrinkled upper surface, sessile apothecia, and an euparaplectenchymatous proper exciple. However, *L. sessile* can be separated by its larger ascospores (16–26 × 10–13 µm in *L. corticola*) and downturned lobe margins.

Key to the non-hairy *Leptogium* species in mainland China

1 Thallus dark brownish black, often with a reddish tinge ..... *L. plicatile*
Thallus grey or blue 2

2 Thallus isidiate 3
Thallus not isidiate 5

3 Isidia cylindrical 4
Isidia squamuliform ..... *L. denticulatum*

4 Thallus pale to dark grayish blue to olive green; isidia granular-furfuraceous, cylindrical to clavate to lobulate, often branched ..... *L. cyanescens*
Thallus grey-blue; isidia terete, simple ..... *L. austroamericanum*

5 Thallus distinctly wrinkled 6
Thallus smooth to slightly roughened 8

6 Thallus with erect to semi-erect lobes, sometimes forming a dense cushion ..... *L. lichenoides*
Thallus with numerous, overlapping lobes forms elegant, spreading patches 7

7 Lobes distinctly thicker (170–450 µm) ..... *L. sessile*
Lobes thinner (100–150 µm) ..... *L. cochleatum*

8 Apothecia distinctly stalked, at the apex of dome-shaped, vertically furrowed (when dry) lobes ..... *L. javanicum*
Apothecia sessile or shortly stipitate 9

9 Spores larger (25–40 × 10–15 µm) ..... *L. subazureum*
Spores smaller (16–25 × 6–13 µm) 10

10 Thallus grey to lead grey, lobes 40–60 µm thick ..... *L. moluccanum*
Thallus blue to blue-grey, lobes 50–100 µm thick ..... *L. azureum*

Fig. 2. ***Leptogium sessile*** (HMAS-L: 031771). A. Thallus with apothecia. B. Thallus cross-section, showing corticated surfaces. C. Hymenium with asci. D. Vertical section of the central apothecium showing euparaplectenchymatous proper exciple with medulla below. E. Thalline margin, showing euparaplectenchymatous proper exciple and thalline exciple. F. Apothecial cross-section. G. Ascus with 8 ascospores. Scale bars: A = 1 cm; B–F = 100 µm; G = 10 µm.

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Regional annotated mycobiotas new to the Mycotaxon website

Abstract — Mycotaxon is pleased to announce another new species distribution list to our 'weblist' page. The "Checklist of the Argentine *Agaricales* 5. *Agaricaceae*" by Niveiro & Albertó brings to 100 the free access mycobiotas now available through the Mycotaxon webpage <http://www.mycotaxon.com/resources/weblists.html>.

SOUTH AMERICA

Argentina

N. Niveiro & E. Albertó. Checklist of the Argentine *Agaricales* 5. *Agaricaceae*. 25 pp.

Abstract — A checklist of species belonging to the family *Agaricaceae* (*Agaricomycetes*) in Argentina is provided. The list includes all species published up to 2011. Of the 13 genera and 171 species represented, *Agaricus* and *Lepiota* have the greatest number of species names recorded (85 and 37 respectively) followed by *Leucoagaricus* (13 species), *Leucocoprinus* (8) *Cystolepiota* (7), *Macrolepiota* (7), and *Cystoderma* (4). The remaining genera — *Cystoagaricus, Micropsalliota, Chlorophyllum, Cystodermella, Pheolepiota* — are represented by fewer than 3 species each.

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